THE BEHAVIOR OF THE CHROMOSOMES IN FERTILIZATION

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The problems of heredity are intimately related to the two critical stages in the life cycle of the higher organisms, the reduction divisions of sporogenesis in plants and of gametogenesis in animals, and the cytological processes of fertilization. The first of these has received much attention and is generally considered to be closely correlated with the segregation of the factors which determine the characters of the next generation. The union of the gametes at the time of fertilization is equally important, although we know little of the behavior of the chromosomes at this period. Recent research has made it desirable from both a cytological and a genetical standpoint to examine critically their behavior in the higher plants.

The behavior of the chromosomes in animals at the time of fertilization is comparatively well known. In some cases the sexual nuclei unite while in the resting condition so that the male and female contribution of chromosomes can not be distinguished. It has been shown, however, that in many species the male and female chromosomes are formed before the fusion of the sexual nuclei and that they may maintain the two distinct groups during the first division of the zygote. In some species the independence of male and female chromosomes has been traced through several divisions and even to later cleavage stages. Indeed there are reasons for believing that the male and female chromosomes may maintain their independence until gametogenesis. In all cases the first division of the fertilized egg appears to be essentially similar to any other normal somatic division.

In plants there is a great deal of variation in the behavior of the sexual nuclei in fertilization. In the algae it has been shown that in some cases the first division of the zygote is a reduction division. In Spirogyra (KARSTEN 1908) it has been found that the first division of the zygospore reduces the chromosome number from twenty-eight to fourteen.

ALLEN (1905) has found that the first division of the oospore nucleus of Coleochaete is also a reduction division. In all cases described the gametes unite completely and male and female chromosomes are not found in separate groups.

The fungi afford an interesting variety of phenomena in connection with fertilization. As HARPER (1910) points out, they have served to enlarge our conception of the sexual process elsewhere. In the rusts a fusion of two cells may occur independently of nuclear fusion as was shown by the work of BLACKMAN (1904) and CHRISTMAN (1905). The nuclei remain separate throughout the sporophytic phase until they fuse in the teleutospore just before reduction. The long period in which the nuclei exist side by side in the sporophyte without fusing and their final fusion prior to reduction is significant. There is no question but that the male and female chromosomes are independent in the sporophytic generation. No less striking are the conditions found in the Ascomycetes. HARPER'S (1905) work on the mildews has shown the existence of two nuclear fusions in the life cycle, one at the origin of the ascocarp where there is a fusion of differentiated gametes, and the other in the ascus. The nuclear fusion in the ascus is followed by three successive divisions which are thought to be correlated with the occurrence of the double fusion.

A most remarkable fusion which takes the place of the normal fertilization has been described in *Aspidium falcatum,* an apogamous fern, by Miss ALLEN (1914). In this case the sporophyte develops through a vegetative bud from the prothallus and has presumably the $1x$ number of chromosomes. Sixteen spore mother cells are present as in other ferns but these fuse in pairs to give eight cells from which a maximum of thirty-two spores may be developed. This fusion sometimes occurs while the spore mother cells are in the spireme stage so that two complete spiremes are present in the fusion nucleus. The fusion is followed immediately by the reduction division characteristic of spore formation.

The behavior of the chromosomes in fertilization has been described for many species of Gymnosperms. It was found by BLACKMAN (1898), FERGUSON (1904), and CHAMBERLAIN (1899), that in Pinus the male and female chromosomes are formed independently for the first division of the fertilized egg. The independent formation of male and female chromosomes has also been described in Tsuga (MURRILL 1900), Juniperus (NORÊN 1907), and Abies (HUTCHINSON 1915). All of these accounts are in general accord with the conditions found in some other plants and in many animals.

A most unusual behavior of the chromosomes in fertilization has been described in *Abies balsamea* by HUTCHINSON (1915). The chromosomes of the male and female gametes are formed independently as is the case in most conifers. As the two groups unite the chromosomes are said to become paired side by side and to twist about one another in a manner similar to their behavior in the reduction divisions. The number of chromosome pairs is haploid. According to HUTCHINSON there is then a *transverse* segmentation of the chromosome pairs. The resulting pairs of chromosomes are diploid in number, and of about half the length of the pairs before segmentation. The chromosomes are described as then separating to form $4x$ chromosomes, half of the number passing to each pole in the first division. The description is not supported by convincing figures.

CHAMBERLAIN (1916) in *Stangeria paradoxa* finds a similar pairing of chromosomes at fertilization. Although he apparently upholds HUTCHINSON'S conclusion he does not state that in this form there is a transverse segmentation of the chromosome pairs in the first division of the egg.

In the angiosperms there is no detailed account of the behavior of the chromosomes during the first division of the fertilized egg. In most cases the sexual nuclei fuse while in the resting condition. Cases have been described in Lilium (GUIGNARD 1891), Cypripedium (PACE 1907), and Fritillaria (SAX 1916), where the gamete nuclei were rarely in the spireme stage before fusion. The first division of the fertilized egg in angiosperms has been described by GUIGNARD (1891), ERNST (1902), GOLDSCHMIDT (1916), RENNER (1914) and the writer (SAX 1916), but in no case have the descriptions been complete and rarely has more than a single division figure been shown in any of the papers.

ATKINSON (1917) in a recent genetical paper on Oenothera has presented some results which he maintains can be explained only on the assumption that there is a segregation of factors in the F_1 zygote. He also states (p. 257):

"The germ plasm is peculiarly sensitive to shock from the meeting of sperm and egg, particularly when there is a genotypic difference between the two germ plasms. This results more or less in interchange, crossing over, dominance, as well as blending, of factors in the zygote, often accompanied by selection of factors into different associations in different zygotes giving rise to more than one hybrid type in the F_1 generation of crosses."

ATKINSON'S statements are largely theoretical, and, as DAVIS (1917)

has indicated, have little experimental and no cytological basis, yet we must consider such possibilities in the present study.

The purpose of this study is to consider not only the behavior of the chromosomes of the gametic nuclei, but also in the "triple fusion." Although it is questionable if we may consider the triple fusion as a real fertilization, yet so far as the inheritance of endosperm characters is involved it is quite comparable to the union of the gametic nuclei. For this reason and also because of the great importance of endosperm characters in cereal breeding, we will give some attention to the chromosomes in the triple fusion in both Fritillaria and Triticum. It is also necessary to describe briefly the development of the embryo sac in Fritillaria in order to understand the chromosome number found in the first division of the endosperm nucleus.

MATERIAL AND METHODS

The material for this paper was secured from about four thousand cases of fertilization in *Fritillaria pudica* Spreng. and not less than two hundred cases of fertilization in *Triticum durum hordeiforme* Hort. var. Kubanka. Flemming's stronger solution and chrom-acetic acid were used as fixatives for the Fritillaria ovaries. At the suggestion of Dr. OSTERHOUT about 0.5 percent of urea salts were added to the above fixatives and gave excellent results with the wheat ovaries. Besides assisting as a fixative, the urea salts reduce the surface tension of the solution and aid in the penetration of the fixative. Sections were cut from 10 μ to 20 μ thick. Modifications of Flemming's triple stain, and Heidenhain's iron alum haematoxylin were used in staining with good results.

THE CHROMOSOMES IN FRITILLARIA

A brief consideration of the chromosome number in the development of the embryo sac in Fritillaria is necessary before describing the later stages. Numerous counts of the chromosome number in the various stages of embryo sac development were made. In the heterotypic division of the megaspore mother cell twelve chromosomes pass to each pole. The second division usually appears to be normal and the four resulting nuclei each receive twelve chromosomes. These nuclei pass into the resting stage. The nuclei in the third division present considerable variation in respect to chromosome number. The two nuclei at the micropylar end of the embryo sac have the usual 1x number of chromosomes and when they divide twelve chromosomes pass to each pole. One of these resulting nuclei becomes the upper polar nucleus. The other

three constitute the nuclei of the cells of the egg apparatus, one of which functions as the egg. One of the two nuclei nearest the chalazal end of the sac in the four-nucleate stage disintegrates. The other, which has received twelve chromosomes from the previous division, has at the time of division not twelve chromosomes, but about twenty-four, and in the division about twenty-four chromosomes pass to each pole. One of the resulting nuclei becomes the lower polar nucleus. As a result of this development of the embryo sac the egg and upper polar nuclei each contain $1x$ or twelve chromosomes while the lower polar nucleus possesses approximately $2x$ chromosomes. The lower polar nucleus and the normal antipodal nucleus are considerably larger than the other nuclei of the embryo sac, presumably because of the $2x$ number of chromosomes which they contain.

The general behavior of the sexual nuclei in Fritillaria has been described in an earlier paper (SAX 1916). The contents of the male nuclei while free in the embryo sac were found to be in an irregular dark-staining network. The male nucleus soon after coming in contact with the egg nucleus loses this netlike structure of its chromatin and passes into the usual resting condition. The union of the sexual nuclei occurs while they are in the resting condition as shown in figure 1, plate 1. The male nucleus can be recognized by its slightly smaller size. In both nuclei there are nucleoli, and the chromatin is in a reticular network. In the cases figured the nuclei are flattened at the point of contact but in some cases the egg nucleus is somewhat indented. The cytoplasm is distributed uniformly throughout the lower part of the egg cell, while the upper part is almost entirely occupied by a large vacuole. The gametic nuclei remain distinct for a comparatively long time, often until the endosperm has reached the four- or eight-nucleate stage.

The disappearance of the adjacent nuclear membranes of the sexual nuclei results in a fusion nucleus which is also in the resting stage. At first there is a trace of the outlines of the two nuclei but later the fusion nucleus shows no indication of its binucleate origin. At this stage all of the chromatin stains alike and it is impossible to distinguish the male and female chromatin as separate contributions. Such a case is shown in figure 2. The chromatin is in the resting condition and more nucleoli are present than in the nucleus of the unfertilized egg.

The fusion nucleus remains in the resting stage but a short time. The chromatin forms a fine threadlike structure and the nucleoli begin to disappear. Figure 3 shows an early spireme stage where the spireme is not completely formed. Delicate threads can be seen among the

heavier chromatin threads. The spireme thread thickens until it appears as illustrated in figure 4. Here the nucleoli have not entirely disappeared and traces of the nuclear membrane may be seen. The broken appearance of the spireme is partly due to the passage of the microtome knife through the edge of the nucleus. But in many other spiremes examined it was also impossible to demonstrate the presence of a single continuous spireme.

After the formation of the spireme the nuclear membrane disappears entirely. The spireme contracts and becomes somewhat thicker just before the segmentation into chromosomes occurs. The segmentation of the spireme is shown in figure 5. The nuclear membrane has completely disappeared and the segmenting spireme has contracted. Surrounding the nuclear cavity is a dense area of cytoplasm from which delicate threads pass to the newly formed chromosomes.

A significant variation in spireme formation of the zygote has been described for Fritillaria in an earlier paper (SAX 1916, fig. 21). In this case the sexual nuclei were found in the spireme stage before fusion. This condition is very rare in the fusion of the egg and male nuclei, but is not uncommon in the nuclei of the triple fusion.

The chromosomes in the metaphase of the first division are long and often cover much of the spindle. The spindle is bipolar and the chromosomes are in a single group and not paired. The number of chromosomes at this time is twenty-four. A longitudinal section of the metaphase of the zygote is presented in figure 6. All of the chromosomes are not shown in this figure, but in this and the two following figures the proportion of chromosomes omitted, due to their presence in an adjacent section, is about the same. The cytoplasm at this time is more uniform than in the preceding stages.

As the chromosomes divide twenty-four pass to each pole. There is no evidence that the male and female chromosomes are separated in two groups. The spindle is typically bipolar. The anaphase of the first division of the zygote is shown in figures 7 and 8. In figure 7 the chromosomes are not so large as in the metaphase. In figure 8 the chromosomes are nearer the poles and they are larger than in the preceding figure. The spindle at the lower end appears to be three-parted, possibly due to the fact that it is cut somewhat obliquely. The other pole has no appearance of being divided. In neither case figured nor in many others examined is there any evidence that the chromosomes pair.

In the telophase the chromosomes form a single compact group at

either pole. The fibers between the poles thicken and the cell plate is formed. A clear telophase is shown in figure 9. Although the chromatin groups are very compact the outlines of the chromosomes are still apparent.

The second division of the embryo appears to be the same as any other normal somatic division. Often one of the cells of the two-celled embryo divides before the other, but usually they divide at the same time.

The second male nucleus and the two polar nuclei usually fuse in the resting condition. Often, however, the nuclei are found in the spireme stage before they fuse and occasionally before the upper polar nucleus and male nucleus have joined the lower polar nucleus. A striking case of the latter condition is shown in figure 10. The nuclei are in early spireme stages. The difference in the size of the nuclei is evident. In this triple fusion the large lower polar nucleus contributes about $2x$ chromosomes while the upper polar nucleus and the male nucleus each contribute $1x$ chromosomes. Thus $3x$ chromosomes are contributed by the maternal parent and only $1x$ by the male parent.

The number of chromosomes in the metaphase of the first division of the "endosperm nucleus" is approximately $4x$ or forty-eight. From figure 11 it is evident that this division appears to be normal. There is no segregation of chromosomes into groups, no pairing of chromosomes, and the spindle is not multipolar.

The chromosomes of the metaphase split longitudinally and about forty-eight daughter chromosomes pass to each pole. In the anaphase shown in figure 12 it was impossible to show all of the chromosomes. Apparently an equal number of chromosomes pass to each pole and the division in every way appears to be regular.

THE CHROMOSOMES IN TRITICUM

The mature embryo sac in wheat consists of the egg cell, two synergids, two polar nuclei in the primordial endosperm, and a large number of antipodal cells. The cytoplasm of the egg cell is more or less vacuolate near the periphery, but a denser layer is found around the nucleus. The egg nucleus contains one large nucleolus and often several small ones. The chromatin is arranged in a loose irregular network. The polar nuclei come in contact with each other, but do not fuse, before fertilization. They are very large and are about equal in size. The cytoplasm of the "Endospermanlage" extends from the polar nuclei to the egg apparatus.

The male nucleus as it enters the egg cell is small, elongated and its

contents are so dense that it appears almost homogeneous in structure. It takes a brilliant red stain with the safranin and is easily recognized. A male nucleus free in the cytoplasm of the egg cell is shown in figure 13. It is long and curved at the ends. The structure of the male nucleus appears almost homogeneous except for small vacuoles and darker-staining particles. There is no trace of a separate cytoplasmic sheath around the male nucleus.

The male nucleus when in contact with the egg nucleus is often more or less coiled, but has the same structure as when free in the egg cell. Such a case is presented in figure 14. Immediately around the male nucleus the cytoplasm is less dense and a few delicate threads cross this vacuolate area. This condition was also found in other preparations. The chromatin of the egg nucleus at this time is still in the resting condition.

The male nucleus apparently penetrates the egg nucleus before increasing in size. Many stages were found where the compact spireme of the male nucleus could be seen inside of the membrane of the egg nucleus. Its appearance is striking and unmistakable because of its density and darker-staining properties. In figure 15 the male nucleus is shown inside of the egg nucleus near the membrane. It has increased in size and the irregular compact spireme thread can be seen. There are several small nucleoli in the male contribution of chromatin. The chromatin of the egg nucleus is in the early spireme stage and portions of the spireme thread are often more or less parallel. In figure 16 the early spireme of the male chromatin is especially distinct. Its dark-staining closely coiled spireme is quite distinct from the loose spireme of the egg nucleus. The nuclear membrane is still apparent at this stage. In both cases figured the large nucleolus of the egg nucleus is shown quite separate from the male chromatin.

The segmentation of the two spiremes of the zygote results in $2x$ chromosomes which are unpaired. The metaphase of the first division of the fertilized egg is illustrated in figure 17. There are approximately twenty-eight chromosomes to be seen in this section. Several pieces of chromosomes, or possibly whole chromosomes, were found in an adjacent section. There is no indication that the chromosomes contributed by the male and female gametes lie in separate groups and they are not in pairs. In several chromosomes shown in this figure a longitudinal division may be seen. This longitudinal splitting of the chromosomes continues until all of the chromosomes have divided, as illustrated in figure 18. There are about twenty-one pairs shown in this figure and

there are six or seven pairs in an adjacent section. These split chromosomes are comparatively long and are often curved. The daughter halves are most widely separated at the center and in some cases they appear to be slightly twisted about one another. After the completion of the longitudinal fission $2x$ daughter chromosomes pass to each pole. An anaphase of the first division is shown in figure 19. The daughter chromosomes are about as long as the mother chromosomes of the metaphase and are often curved. The spindle is clearly bipolar. The dense layer of cytoplasm so conspicuous about the egg nucleus in the earlier stages is apparent around the spindle.

The chromosomes pass to each pole in a single group. In figure 20 approximately twenty-eight chromosomes may be counted at each pole in the late anaphase. The chromosomes have shortened a little and are in compact groups. It will be noted that the dense area of cytoplasm in the egg cell is for the most part around only the upper pole, while the chromosomes of the lower pole lie in the more vacuolate cytoplasm. The chromatin of the telophase passes into the resting stage, a cell plate is laid down and the two-celled embryo is formed.

The male nucleus which unites with the polar nuclei appears to be quite similar to the one which unites with the egg nucleus. In figure 21 the triple fusion is shown. The dark-staining male nucleus is coiled at the side of one of the polar nuclei. The polar nuclei are large and of about equal size. Each contains a large nucleolus and the chromatin is in granules in an open network.

At the time of spireme formation the chromatin contributed by each of the three nuclei may be distinguished. Such a case is shown in figure 22. Here the spireme of the male nucleus is in a comparatively compact ball at the upper side of the figure. The spireme of each polar nucleus is rather open, very irregular, and quite distinct. The nucleolus and outlines of each polar nucleus may be seen, but the nuclear membranes have almost entirely disappeared.

The chromatin of the male nucleus and polar nuclei maintain their independence not only in the spireme stage, but apparently also in the early division stages. Figures 23 A and 23 B are drawn from adjacent sections of the first division of the triple fusion nucleus. This is the early metaphase when the chromosomes are on the nuclear plate. There are approximately forty chromosomes to be counted. The chromosomes appear to be in three groups, each group containing approximately an equal number of chromosomes. The spindle in figure 23 A is clearly two-parted at the upper end and more or less so at the lower end. The

spindle in figure 23 B appears to be distinct from the spindles in figure 23 A. Later stages in the first division of the triple fusion nucleus were not observed, but judging from many counts of later endosperm divisions, the first division is regular and an equal number of chromosomes pass to each pole. The fact that there are about forty chromosomes in the endosperm divisions indicates that the polar nuclei each contribute $1x$ chromosomes. After the first division the endosperm nuclei divide rapidly and cell walls are not formed in the early development of the endosperm.

DISCUSSION

In many animals and in most gymnosperms the independence of the formation of the male and female chromosomes following fertilization is unquestioned. There are a few cases in the angiosperms where the independent formation in the zygote of the spiremes from the gametes has been demonstrated. In an earlier paper (SAX 1916) the writer described the gamete nuclei of Fritillaria, in rare cases, as in the spireme stage before fusion. In wheat the spireme of the male and female nuclei are formed separately even though the male nucleus is within the egg nucleus at the time of spireme formation (figures 15 and 16). In both Fritillaria and Triticum the nuclei of the triple fusion may be in the spireme stage before fusion. In Triticum the chromosomes contributed by each nucleus appear to be in more or less separate groups even in the metaphase of the first division. Although there is considerable variation in the condition of the sexual nuclei at the time of fusion, I believe that we are justified in assuming that the male and female chromosomes are formed independently in the zygote, even in cases where the nuclei fuse in the resting condition.

The behavior of the chromosomes during the first division of the egg in Fritillaria and Triticum is essentially not different from any other normal somatic division. There is no indication that the chromosomes of the male and female gametes are in separate groups, or that they pair in the first division of the zygote. In Triticum the chromosomes in the metaphase, when first split longitudinally, appear much like certain stages in Abies, which HUTCHINSON (1915) interprets as a pairing of chromosomes. But in Triticum the chromosomes of the metaphase before splitting are distinctly $2x$ in number and after splitting there are $2x$ pairs. If a pairing of male and female chromosomes occurred we would expect to find only $1x$ pairs of chromosomes.

In neither Triticum nor. Fritillaria is there anything comparable to

a reduction division in the fertilized egg nor are there any irregularities which might account for a zygotic segregation of factors. It is possible, as ATKINSON (1917) suggests, that there is a "shock" as the result of the meeting of genotypically different germ plasms, which may cause an irregular behavior of the first division of the zygote. The only cytological work which might support this theory is that of GOLDSCHMIDT (1916). He explains the occurrence of patrocliny in Oenothera crosses as a result of merogony. There is no good cytological evidence of any behavior which would explain a segregation of factors during the first division of the zygote. The segregation of factors in the F_1 as described by ATKINSON, might be accounted for on the assumption that in Oenothera the chromosomes of the zygote behave as described in Abies. But this assumption would lead into other problems more difficult to explain. It is much more probable, as DAVIS (1917) has suggested, that ATKINSON is dealing with genotypically impure parent species, and that his assumptions concerning the segregation of factors in the zygote are entirely unnecessary.

The behavior of the chromosomes of the zygote in Abies, as described by HUTCHINSON (1915), if verified, would be of considerable genetical significance to those who consider the chromosomes the bearers of the hereditary factors. According to HUTCHINSON the chromosomes of the male and female nuclei pair in the zygote so that there are $1x$ pairs. Each pair segments transversely, forming $2x$ pairs of chromosomes. The chromosome segments separate and $2x$ chromosomes pass to each pole. There is no longitudinal division of the chromosomes as is the case in the usual type of somatic division. If we assume that the hereditary factors are located in the chromosomes it is obvious that if homologous chromosome segments pass to opposite poles the hereditary factors will be segregated according to the usual Mendelian behavior. We would then expect a segregation of characters in F_1 individuals in crosses when the parents differ in Mendelizing characters. If we assume that not only are the hereditary factors located in the chromosomes, but also in a definite linear arrangement in the chromosomes, it is evident that the chromosomes can not divide longitudinally at one time and transversely at another time, as described in Abies, without causing chaos in the distribution of the hereditary factors. Furthermore if this process continued through several generations each chromosome would finally contain but a single factor and most of the factors would be lost. Certainly the behavior of the chromosomes in Abies, as

described by HUTCHINSON, can not be brought into harmony with the theory of a linear arrangement of the factors in the chromosomes. To be sure the latter theory is only a working hypothesis, but the research of MORGAN and his students, and other experimental work, can best be explained by it.

If in Abies there is a double "reduction," one at the time of sporogenesis, the other during the first division of the zygote, we might expect a compensating fusion at some period in the life cycle. A comparable case is found in the mildews where the double fusion is thought to be followed by a double reduction in the ascus. In view of the present study and the theoretical questions raised by HUTCHINSON'S results, it would be well to examine more critically the behavior of the chromosomes in the first division of the zygote in Abies.

The pairing of the chromosomes in somatic cells has been described in Galtonia, Funkia, Oenothera, Thalictrum and Yucca. It appears that the pairing of the chromosomes may take place at the time of fertilization, as HUTCHINSON maintains is the case in Abies and as CHAMBERLAIN (1916) has described in Stangeria, or that the pairing may not occur until synapsis as is true in the rusts. There may be intermediate examples where the chromosomes pair at various times in the sporophytic life cycle. It is possible as Miss FRASER (1912) suggests, that "the clearest cases of Mendelian inheritance will perhaps be those correlated with a late association of the chromosomes in pairs." Experimental work, however, makes it appear probable that the chromosomes are quite independent, and that if there is any influence or interchange of factors between chromosomes it does not occur until synapsis. According to HARPER (1910) the behavior of the chromosomes in the rusts "is certainly strongly suggestive that synapsis and its accompanying phases represent a stage of mutual influence if not of interchange of physical material between the chromosomes much more intimate than any which has preceded it in the life of the sporophyte."

In Fritillaria and in Triticum there is no evidence that the chromosomes pair in fertilization. The male and female spiremes are formed independently. At synapsis there is a pairing of male and female chromosomes. We might expect that here two spiremes are formed, one male and one female, as in fertilization, and that these spiremes or portions of these spiremes, pair side by side (parasynapsis), rather than expect that homologous chromosomes become arranged alternately end to end and then pair as in telosynapsis. In Aspidium where the

spore mother cells fuse while in the spireme stage there is a reduction division immediately following. In this case there is probably a pairing of homologous chromosomes as is generally thought to occur in reduction divisions. It is more difficult to assume that here the chromosomes should follow the telosynaptic method of reduction than to assume that the spiremes conjugate side by side as in parasynapsis. In either case, however, the physical difficulties seem to be very great. If there is a somatic pairing of chromosomes in cases where the telosynaptic method of reduction occurs we might expect the male and female chromosomes to become arranged alternately end to end at or soon after fertilization.

In the development of the embryo sac in Fritillaria twenty-four chromosomes are found in the metaphase of the third division at the chalazal end of the embryo sac, and as a result of this division the lower polar nucleus receives about $2x$ or twenty-four chromosomes. A similar doubling of the chromosome number at the chalazal end of the embryo sac has been described in *Lilium martagon* by GUIGNARD (1891), MOTTIER (1897), SARGANT (1896), and STRASBURGER (1908). According to STRASBURGER this doubling is due to a premature longitudinal splitting of the chromosomes and is dependent on the food supply of the plant. In Fritillaria the lower polar nucleus which contains about $2x$ chromosomes is considerably larger than the upper polar nucleus which contains $1x$ chromosomes. Since both nuclei are surrounded by a common cytoplasm, the difference in size is probably due to the difference in chromosome number. In Triticum the polar nuclei are of equal size and so probably have the same chromosome number, presumably $1x$ each. The difference in size between the polar nuclei and the egg nucleus is apparently due to the nuclear cytoplasmic relations.

In the triple fusion in Fritillaria there are about $3x$ chromosomes contributed by the female parent and $1x$ chromosomes contributed by the male parent. If we assume that the hereditary factors are borne by the chromosomes, then the female parent contributes three sets of factors and the male parent contributes only one set. This phenomenon is of considerable importance in relation to the multiple factor hypothesis. For instance HAYES and EAST (1915) have found in a particular cross in corn that the endosperm character borne by the mother was always dominant. One of the parents used had a starchy endosperm, the other a corneous endosperm. Either way the cross was made the F_1 endosperm was always of the same kind as the endosperm of the mother parent. This type of inheritance is explained on the assumption that the

two factors contributed by the mother, as a result of polar fusion, are always dominant over the single factor contributed by the male parent. The effect of multiple factors is clearly demonstrated here, and if the female parent contributes not two, but three factors, the maternal contribution should be still more potent.

It is unnecessary to consider the morphological character of the endosperm because this subject has been dealt with fully by EAST (1913). It may be added, however, that the development of the endosperm is independent of the development of the embryo. While working with Mr. E. F. GAINES at WASHINGTON STATE COLLEGE, I found about a dozen cases in wheat where the endosperm had developed normally and yet no embryo was present. One such case has been found in corn. Such a condition, although very rare, is of theoretical interest.

The significance of double fertilization has been a puzzle to many botanists. The suggestion of NEMEC (1910) seem to be the best explanation and has some experimental proof. NEMEC maintains that an endosperm whose characters are contributed by both parents is better suited to the nourishment of the embryo than an endosperm of wholly maternal origin. STINGL'S (1907) work supports this theory. He has transferred embryos of various cereals to their own and other endosperms. In most cases the embryo grew better on its own endosperm than on that of another genus. Preliminary experiments of my own in transferring hybrid corn embryos to the parent endosperms and *vice versa,* have not as yet given definite results. In view of STINGL'S results and my own work it appears that the experimental error is too large to permit small differences in adaptability of embryo and endosperm to be noted. If we assume that a hybrid endosperm is better able to nourish the hybrid embryo than an endosperm derived from the mother only, we might expect that "wider" crosses could be made among plants where the endosperm is derived from both parents.

CONCLUSIONS

A complete series of stages in the first division of the fertilized egg has been found in both *Fritillaria pudica* Spreng. and in *Triticum durum hordeiforme* Host. var. Kubanka.

In Fritillaria the male and female nuclei are of about equal size before fusion. The sexual nuclei usually unite while in the resting condition, although occasionally they are in the spireme stage at the time of fusion. The presence of a single continuous spireme in the zygote

could not be demonstrated at any time. In the first division of the fertilized egg each chromosome contributed by the male and female gametes splits longitudinally and twenty-four chromosomes pass to each pole.

In Triticum the male nucleus is small and almost homogeneous in structure even when in contact with the egg nucleus. The male nucleus enters the egg nucleus and forms a separate compact spireme. At the same time the spireme of the egg nucleus is formed. In the first division approximately twenty-eight chromosomes are found in the metaphase. These chromosomes split longitudinally and the $2x$ number pass to each pole.

In both Fritillaria and Triticum the first division of the zygote appears to be essentially like any other normal somatic mitosis. The male and female chromosomes are formed independently; they are not found in separate groups, nor do they pair. There is no chromosome behavior at this time which might account for a zygotic segregation of factors.

In the triple fusion in Fritillaria the nuclei are often found in the spireme stage before fusion. The first division of the endosperm nucleus appears to be very regular. The chromosomes are not paired and there is no segregation of the chromosomes contributed by each nucleus. In this division about $4x$ or forty-eight chromosomes pass to each pole. This number is due to the fact that there is a doubling of the chromosomes at the chalazal end of the embryo sac in the third division and as a result the lower polar nucleus receives about twenty-four chromosomes. Thus in the triple fusion the female parent contributes $3x$ chromosomes and the male parent contributes only $1x$ chromosomes. This phenomenon is of interest in relation to the multiple factor hypothesis.

In Triticum the nuclei involved in the triple fusion form separate spiremes. There is evidence that the chromosomes contributed by each nucleus may remain more or less separate even in the metaphase of the first division. Each nucleus contributes $1x$ or about fourteen chromosomes.

In both Fritillaria and Triticum the chromosomes of the triple fusion are formed independently, there is no pairing of chromosomes and the first and following divisions appear to be regular.

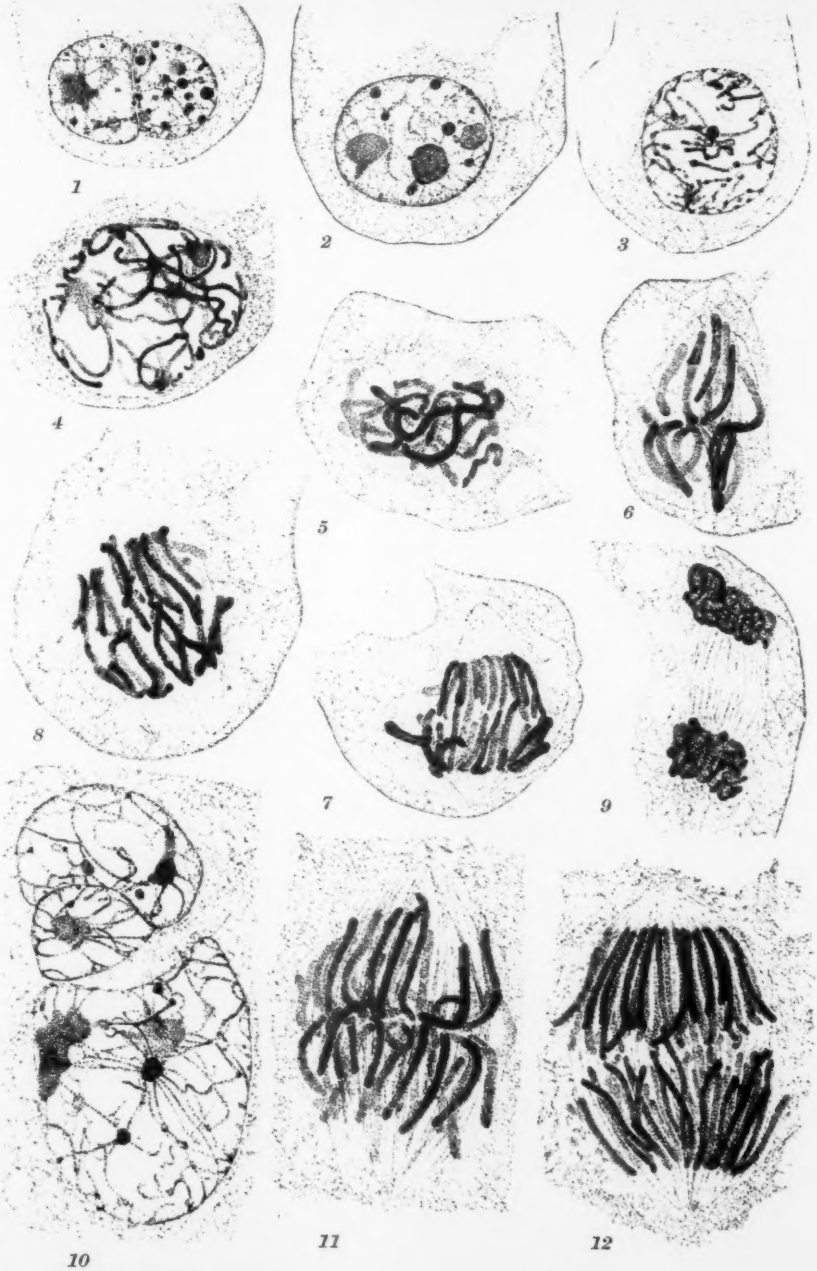
This work was done under the direction of Dr. E. M. East to whom I am indebted for suggestions and criticisms. I am also indebted to my wife for much assistance.

EXPLANATION OF PLATES

All drawings were made with the aid of the camera lucida. The figures were drawn from single sections with the exception of figure 22. The magnification is as follows: Figure 3, 1120 diameters; figure 21, 900 diameters; all other figures, 1200 diameters. No reduction.

PLATE 1.—FRITILLARIA

FIGURE 1.—Male nucleus and egg nucleus in contact.
FIGURE 2.—Complete fusion of male and egg nuclei.
FIGURE 3.—Early spireme of fertilized egg.
FIGURE 4.—Spireme of fertilized egg.
FIGURE 5.—Segmentation of spireme. Formation of chromosomes.
FIGURE 6.—Metaphase of the first division of the fertilized egg.
FIGURES 7, 8.—Anaphase of the first division of the fertilized egg.
FIGURE 9.—Telophase of the first division of the fertilized egg.
FIGURE 10.—Early triple fusion.
FIGURE 11.—Metaphase of the first division of the endosperm nucleus.
FIGURE 12.—Anaphase of the first division of the endosperm nucleus.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LITERATURE CITED

ALLEN, C. E., 1905 Die Keimung der Zygote bei Coleochaete. Ber. d. d. bot. Gesell. **23**: 285-292, *Taf. 13.*

ALLEN, R. F., 1914 Studies in spermatogenesis and apogamy in ferns. Trans. Wisconsin Acad. Sci. **17**: 1-56, *pls. 1-6.*

ATKINSON, G. F., 1917 Quadruple hybrids is Oenothera. Genetics **2**: 213-260.

BLACKMAN, V. H., 1898 On the cytological features of fertilization and related phenomena in *Pinus silvestris* L. Phil. Trans. Roy. Soc. **190**: 395-426, *pls. 12-14.*

1904 On the fertilization, alternation of generations and general cytology of the Uredineae. Ann. Bot. **18**: 323-373, *pls. 21-24.*

CHAMBERLAIN, C. J., 1899 Oogenesis in *Pinus laricio.* Bot. Gaz. **27**: 268-280, *pls. 4-6.*

1916 *Stangeria paradoxa.* Bot. Gaz. **61**: 353-372, *pls. 24-26.*

CHRISTMAN, A. H., 1905 Sexual reproduction in the rusts. Bot. Gaz. **39**: 267-275, *pl. 8.*

DAVIS, B. M., 1917 The test of a pure species of Oenothera. Proc. Amer. Phil. Soc. **54**: 226-245.

EAST, E. M., 1913 Xenia and the endosperm of angiosperms. Bot. Gaz. **56**: 217-224.

ERNST, A., 1902 Chromosomenreduction, Entwicklung des Embryosacks und Befruchtung bei *Paris quadrifolia* L. und *Trillium grandiflorum* Salisb. Flora **91**: 1-46, *Taf. 1-6.*

FERGUSON, M. C., 1904 Contributions to the life history of Pinus. Proc. Washington Acad. Sci. **6**: 1-202, *pls. 1-24.*

FRASER, H. I. C., 1912 The pairing of the chromosomes. New Phyt. **11**: 58-60.

GOLDSCHMIDT, R., 1916 Nochmals über die Merogonie der Oenotherabastarde. Genetics **1**: 348-353, *pl. 4.*

GUIGNARD, L., 1891 Nouvelles études sur la fécondation. Ann. Sci. Nat. Bot. **14**: 163-296, *pls. 9-18.*

HARPER, R. A., 1905 Sexual reproduction and the organization of the nucleus in certain mildews. Carnegie Inst. Washington. Publ. 37. 92 pp., *7 pls.*

1910 Nuclear phenomena of sexual reproduction in fungi. Amer. Nat. **44**: 595-603.

HAYES, H. K. and EAST, E. M., 1915 Further experiments on inheritance in maize. Conn. Agr. Exp. Sta. Bull. 188. pp. 1-31, *pls. 1-7.*

HUTCHINSON, A. H., 1915 Fertilization in *Abies balsamea.* Bot. Gaz. **60**: 457-472, *pls. 16-23.*

KARSTEN, G., 1908 Die Entwicklung der Zygoten von *Spirogyra jugalis* Ktzg. Flora **99**: 1-11, *Taf. 1.*

MOTTIER, D. M., 1897 Ueber das Verhalten der Kerne bei der Entwicklung des Embryosacs. Jahrb. f. wiss. Bot. **31**: 125-158, *Taf. 2, 3.*

MURRILL, W. A., 1900 The development of the archegonium and fertilization in the hemlock spruce (*Tsuga canadensis*). Ann. Bot. **14**: 583-607, *pls. 31, 32.*

NEMEC, B., 1910 Das Problem der Befruchtungsvorgänge und andere zytologische Fragen. 532 pp. *5 Taf.* Berlin: Gebrüder Borntraeger.

NORÉN, C. O., 1907 Zur Entwicklungsgeschichte des *Juniperus communis.* Upsala Univ. Årsskr. 64 pp., *4 pls.*

OVERTON, E., 1893 Über die Reduktion der Chromosomen in den Kernen der Pflanzen. Vierteljahrsschr. Naturf. Gesell. Zurich **38**: 169-186.

PACE, L., 1907 Fertilization in Cypripedium. Bot. Gaz. **44**: 353-374, *pls. 24-27.*

PLATE 2.—TRITICUM

FIGURE 13.—Male nucleus in the egg cell.
FIGURE 14.—Male nucleus in contact with the egg nucleus.
FIGURES 15, 16.—Male nucleus within the egg nucleus.
FIGURE 17.—Metaphase of the first division of the fertilized egg.
FIGURE 18.—Late metaphase of the first division of the zygote.
FIGURE 19.—Early anaphase of the first division of the zygote.
FIGURE 20.—Late anaphase of the first division of the zygote.
FIGURE 21.—Early triple fusion.
FIGURE 22.—Spireme stage in the triple fusion.
FIGURES 23A, 23B.—Adjacent sections of the early metaphase of the first division of the triple fusion nucleus.

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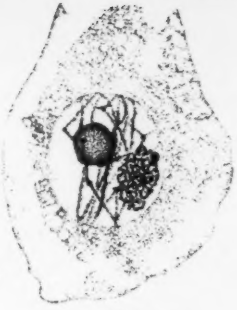

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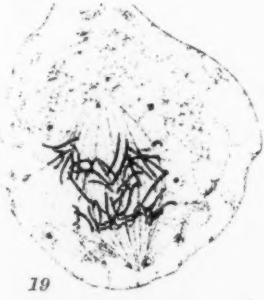

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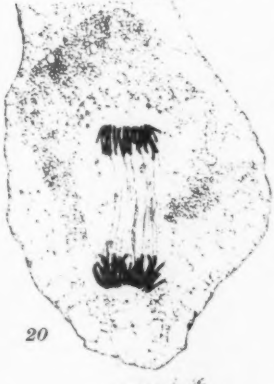

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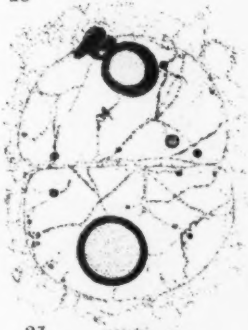

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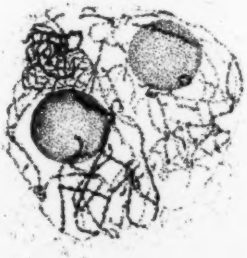

22

23a

23b

RENNER, O., 1914 Befruchtung und Embryobildung bei *Oenothera Lamarckiana* und einigen verwandten Arten. Flora **107**: 115-150, *Taf. 12, 13.*

SARGANT, E., 1896 The formation of the sexual nuclei in *Lilium martagon.* Ann. Bot. **10**: 443-477, *pls. 32, 33.*

SAX, K., 1916 Fertilization in *Fritillaria pudica.* Bull. Torrey Bot. Club **43**: 505-522, *pls. 27-29.*

STRASBURGER, E., 1908 Chromosomenzahlen, Plasmastrukturen, Vererbungsträger und Reducktionsteilung. Jahrb. f. wiss. Bot. **45**: 479-570, *Taf. 1-3.*

STINGL, G., 1907 Experimentelle Studie über die Ernährung von Pflanzlichen Embryonen. Flora **97**: 308-331.

FURTHER ILLUSTRATIONS OF THE APPLICABILITY OF A COEFFICIENT MEASURING THE CORRELATION BETWEEN A VARIABLE AND THE DEVIATION OF A DEPENDENT VARIABLE FROM ITS PROBABLE VALUE

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TABLE OF CONTENTS

PAGE

INTRODUCTORY 328
Earlier applications 331
Further illustrations 332
Illustration 1. Proportionality of parts in Paramecium 332
Illustration 2. Absence of relationship between size of litter and sex in swine 336
Illustration 3. Proportion of pistillate and hermaphrodite flowers in the inflorescence of the composite Homogyne 336
Illustration 4. Fertility of capsules and viability of seed in carnation crosses 337
Illustration 5. Relationship between the total number of pedicels and the number of abnormal pedicels in *Spiraea Vanhouttei* 337
Illustration 6. Interrelationship of cotyledons and primordial leaves in a race of *Phaseolus vulgaris* highly variable in seedling characters 340
Illustration 7. Change in proportion of parts in developing trout 344
Illustration 8. Relationship between total solids and sucrose content in the juice of sugar beets 346
Illustration 9. Relationship between total number of spikelets and number of sterile spikelets in wheat 348
Illustration 10. Viability of dominants and recessives in F_2 generation of Mendelian hybrids 349
RECAPITULATION 350
LITERATURE CITED 351

INTRODUCTORY

Eight years ago I pointed out (HARRIS 1909 a) that when y is some fraction of x the correlation between them, r_{xy}, while of descriptive value,

does not give all of the information which is required concerning the interrelationship of these two variables, and that a coefficient showing whether the value of y becomes relatively larger or smaller with increasing values of x, would have considerable analytical value.

I showed[1] then that if $z = px$, where $p = \bar{y}/\bar{x}$, the bars denoting population means,

$$r_{xz} = \frac{r_{xy} - v_x v_y}{\sqrt{1 - r_{xy}^2 + (r_{xy} - v_x/v_y)^2}}.$$

The purpose of this paper is to illustrate the range of usefulness of this coefficient by noting biological progress which has been made by its use, and by actually applying it to series of data which have not been heretofore fully analyzed by the higher statistical methods.

Before passing to actual illustrations of applicability of the coefficient, some questions of method should be considered.

In cases in which the coefficient r_{xz} is desired the correlation table has usually been formed to determine r_{vy}, where $v + y = x$. The mean and standard deviation for x made up of any number of components (HARRIS 1917 c) are well known (PEARL 1909, HARRIS 1918 a); when $x = v + y$

$$\bar{x} = \bar{v} + \bar{y}, \qquad \sigma_x = \sqrt{\sigma_v^2 + \sigma_y^2 + 2r_{vy}\sigma_v\sigma_y}$$

If one has made his summations about zero as origin, as suggested elsewhere (HARRIS 1910 b) it is quite easy to determine the necessary moments from those already available.

$$\bar{x} = \Sigma(x)/n = [\Sigma(v) + \Sigma(y)]/n$$
$$\sigma_x^2 = [\Sigma(v^2) + \Sigma(y^2) + 2\Sigma(vy)]/n - \bar{x}^2$$

The product moments are

$$\Sigma(xv) = \Sigma(v^2) + \Sigma(vy)$$
$$\Sigma(xy) = \Sigma(y^2) + \Sigma(vy)$$

$\Sigma(vy)$ is in each case the product moment already obtained in the calculation of r_{vy}.

In the original paper (HARRIS 1909 a), the method of determining the value of σ_z, which is essential for calculating the equation for the regression of z on x, was not indicated.

[1] The deduction of a usable formula for the problem then in hand was, as I have elsewhere pointed out, due to Professor PEARSON who, with characteristic generosity to his pupils, insisted that my name only appear in the paper.

The first and second moment of y for each grade of x, i.e., $\Sigma(y_x)$ and $\Sigma(y_x^2)$, are easily determined. The first of these will have been obtained in computing the population product moment, $S(xy)$, by the method cited above.

Remembering that $\Sigma(y_x)$ and $\Sigma(y_x^2)$ are the first and second moments about zero as origin for the several y arrays of x, the moments of z for each array are

$$\Sigma(z_x) = \Sigma(y_x) - n_x px$$

From these the means of arrays which are required for plotting the regression curve are given at once by

$$\bar{z}_x = [\Sigma(y_x) - n_x px]/n_x$$

For the whole population the first moment of z about o as origin is

$$S\Sigma(z_x) = S[\Sigma(y_x) - n_x px] = 0$$

The second moments for the individual arrays are

$$\Sigma(z_x^2) = \Sigma(y_x^2) - 2px[\Sigma(y_x) - n_x px] - n_x(px)^2,$$

and for the population

$$S\Sigma(z_x^2) = S\{\Sigma(y_x^2) - 2\,px[\Sigma(y_x) - n_x px] - n_x(px)^2\},$$

or in some cases more conveniently for actual computation,

$$S(z^2) = S(y^2) - 2pS(xy) + S[n_x(px)^2],$$

where S denotes summation of values of y arrays of x, or throughout the population. Thus

$$\sigma_z = [S(z^2)/N]^{1/2}$$

It is quite possible to determine the correlation between x and z, the deviation of y from its probable value, directly.

Remembering that $[\Sigma(y_x) - n_x px]$ is the first moment about zero as origin of z for any array, the product moment for the population is

$$Sx[\Sigma(y_x) - n_x px],$$

where S denotes summation of the values of the y arrays of x.

Practically it is more convenient to determine the product moment from

$$S(xz) = S(xy) - pS(x^2),$$

where $S(xy)$ and $S(x^2)$ are the product moments of x and y and the second moment of x for the population.

EARLIER APPLICATIONS

The method has been most extensively applied to problems of fertility and fecundity. Thus the relationship between the number of ovaries formed and the number of ovaries developing into fruits has been investigated in the inflorescence of Staphylea (HARRIS 1909), Celastrus (HARRIS 1909 b) and Crinum (HARRIS 1912). In Staphylea and Crinum inflorescences which produce larger numbers of flowers mature relatively fewer fruits. In Celastrus there is apparently no relationship between the number of flowers formed and the capacity of the inflorescence for maturing the ovaries into fruits.

In the fruit, the relationship between the total number of ovules laid down and the deviation of the number of seeds matured from their probable number has been investigated in Sanguinaria (HARRIS 1910 a).

For *Phaseolus vulgaris* a first study (HARRIS 1913) of 53 series comprising 166,130 pods and a supplemental investigation of 16 series comprising 56,698 pods (HARRIS 1917 a) leave no doubt that the pods with the larger number of ovules mature relatively fewer of their ovules into seeds. The same relationship holds in the arborescent legume, *Cercis canadensis*, as is shown by studies based on massed data (HARRIS 1914 a) and on series from individual trees (HARRIS 1914 b).

The relationship found in Cercis and Phaseolus is not universal for the Leguminosae. In a series of 1427 pods of Robinia (HARRIS 1909 a), the pods with larger numbers of ovules mature a relatively higher proportion of their ovules into seeds. The correlation between the actual number of ovules formed and the actual number of seeds developing is $r_{os} = .693 \pm .009$, while that between the number of ovules formed and the deviation of the number of seeds matured from their probable value is $r_{ox} = .365 \pm .015$.

That this result represents a real biological relationship is indicated by the correlations, hitherto unpublished, for the individual trees. Only three of the twelve constants in table 1 are negative in sign. No one of these can be regarded as statistically significant when the probable error is taken into consideration, while seven of the nine positive coefficients must be looked upon as statistically trustworthy.

The formula has also been advantageously applied to the problem of the interrelationship of the number of male and female flowers in the inflorescence of the aroid Arisarum (HARRIS 1916 a) and that of the interdependence of numbers of stamens and pistils in the ranunculaceous genus, Ficaria (HARRIS 1918). In Arisarum the relative number

TABLE I

Relationship between seed and ovule number in Robinia.

Number of tree	Number of pods	r_{os}	r_{oz}	$r_{oz}/E_{r_{oz}}$
1	122	.524±.044	—.055±.061	(—)0.90
2	64	.802±.030	.468±.066	7.09
3	111	.478±.049	.074±.064	1.16
4	102	.430±.054	.105±.066	1.59
5	122	.671±.034	.338±.054	6.26
6	120	.533±.044	.272±.057	4.77
7	120	.259±.057	—.115±.061	(—)1.89
8	159	.614±.033	.216±.051	4.24
9	128	.590±.040	.350±.052	6.73
10	78	.507±.057	.225±.073	3.08
11	105	.714±.032	—.044±.066	(—)0.67
12	196	.797±.018	.489±.037	13.22
All trees	1427	.693±.009	.365±.015	24.33

of pistillate flowers increases as the total number of flowers per inflorescence increases. In Ficaria the relative number of pistils increases as the total number of sporophylls becomes larger.

Dr. BLAKESLEE and I (1918) have applied this coefficient to the determination of the relationship between the total annual egg production and the monthly egg production of White Leghorn fowl. We have there shown by means of this coefficient that the winter months, November, December, January and February, and the following autumn months, August, September and October, show an increase over their theoretical quota of eggs when the annual total egg production rises above the normal. That is, r_{ez}, the correlation between total annual egg production and the deviation of the monthly production from its probable value, is on the whole significantly and substantially positive. The spring and summer months, April, May, June and July, show negative values of r_{ez}, that is, they make a lower relative contribution to the annual total than might be expected when the total varies in the direction of an increase above the normal egg production of the flock as a whole.

FURTHER ILLUSTRATIONS

Illustration 1. Proportionality of parts in Paramecium

JENNINGS (1911) in his masterly investigation of assortative mating in Paramecium, has given data for determining the relationships between

(*a*) distance from the anterior end of the organism to the posterior margin of the mouth, (*b*) distance from the posterior margin of the mouth to the posterior end of the organism, and (*c*) the total length in series of conjugant and non-conjugant Paramecia.

He has calculated and discussed for a purpose which does not concern us here the correlations between certain of these dimensions. All the correlations between the absolute measurements, calculated from his data, are given in table 2.

TABLE 2

Relationship between total length, l, and anterior length, a, and between anterior length and posterior length, p, in Paramecium.

Series*	r_{ap}	r_{la}	r_{lz}	$r_{lz}/E_{r_{lz}}$
Lot 7, table 40	.246±.050	.735±.025	—.733±.025	3.0
Lot 7, table 41	.570±.047	.893±.014	—.427±.057	7.5
Lot 19, table 51	.382±.044	.832±.016	—.434±.042	10.4
Lot 19, table 52	.671±.034	.939±.074	—.196±.060	3.3
Lot 22, table 55	—.403±.026	.485±.049	—.282±.060	4.7
Lot 22, table 56	.620±.025	.906±.073	—.405±.034	12.0
Lot 24, table 64	.277±.040	.820±.014	—.282±.040	7.1
Lot 24, table 65	.488±.031	.885±.089	—.288±.038	7.6

* Lot 7, table 40, conjugants of wild cultures. 2. Lot 7, table 41, non-conjugants of wild cultures. 3. Lot 19, table 51, conjugants of race *g*. 4. Lot 19, table 52, non-conjugants of race *g*. 5. Lot 22, table 55, wild culture conjugants not yet separated. 6. Lot 22, table 56, wild culture conjugants about twelve hours after separation. 7. Lot 24, table 64, unseparated conjugants of race *k*. 8. Lot 24, table 65, conjugants of race *k* about twelve hours after separation.

In this table the constants for anterior and posterior length are arranged in pairs of conjugants and non-conjugants or ex-conjugants. In every instance the correlation between the anterior and posterior portions of conjugants is lower than that between the same dimensions in non-conjugants or ex-conjugants.[2]

All of these values are low, as JENNINGS has noted. In the case of lot 22 the coefficient for the conjugants is actually negative in sign.

The correlation between total length and the length of the section anterior to the mouth is high. In every case the value of r_{la} is higher in non-conjugants or ex-conjugants than in conjugants. The additional relationship to be brought out by the formulae here under discussion is

[2] See in this connection the discussion by JENNINGS (1911, pp. 65-66, 71-73).

that between the total length of the organism and the relative length of either anterior or posterior element.

JENNINGS (1911, p. 63) has emphasized the high variability of the post-oral dimension. Table 3, in which all the coefficients of variation

TABLE 3

Coefficients of variation for anterior and posterior fractions of length in Paramecium.

Series	Total length	Anterior length	Posterior length	$V_p - V_a$
Lot 7, table 40	6.90	5.79	13.86	+8.07
Lot 7, table 41	9.68	8.76	14.53	+5.77
Lot 19, table 51	8.55	7.78	14.31	+6.53
Lot 19, table 52	12.18	12.08	15.13	+3.05
Lot 22, table 55	5.48	7.38	14.29	+6.91
Lot 22, table 56	7.96	7.28	11.20	+3.92
Lot 24, table 64	6.34	6.42	9.98	+3.56
Lot 24, table 65	6.62	6.45	9.35	+2.90

are laid side by side, fully confirms his conclusion in this regard. Utilizing these coefficients of variation we obtain the values for the correlation between total length and the deviation of the anterior length from its probable value, given in the fourth column of table 2.

These constants are negative in sign throughout, and while variable in magnitude all may reasonably be considered statistically significant in comparison with their probable errors.

Thus when Paramecium varies in length both anterior and posterior fractions of the body contribute to this variation, but as length increases the anterior portion becomes relatively shorter.

For one series, the unseparated conjugants of race *k*, I have determined the regression of the anterior length on total length and the regression of the deviation of the anterior length from its probable value on the total length of the organism. The equations are

$$a = 3.0359 + .4977\, l$$
$$\sigma_x = .7616, \quad z_a = 3.3743 - .1135\, l$$

The equations and empirical means are represented graphically in diagrams 1 and 2. In both cases the relationships are sensibly linear.

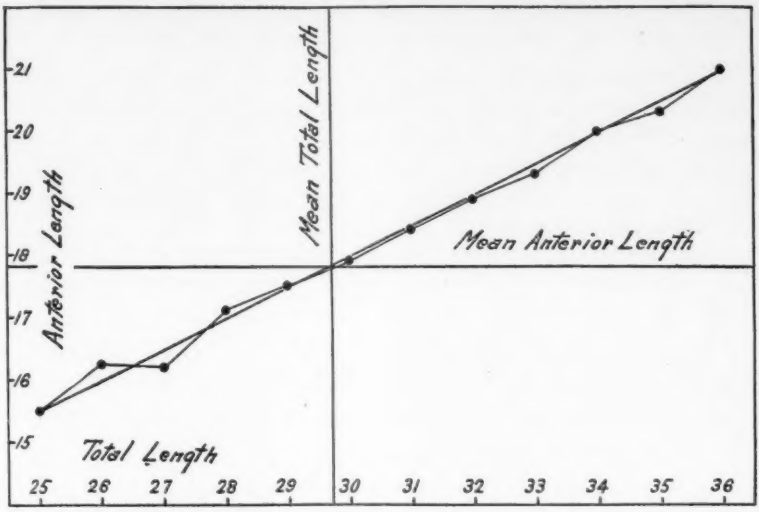

DIAGRAM 1.—Relationship of anterior length to total length in Paramecium. Compare diagram 2.

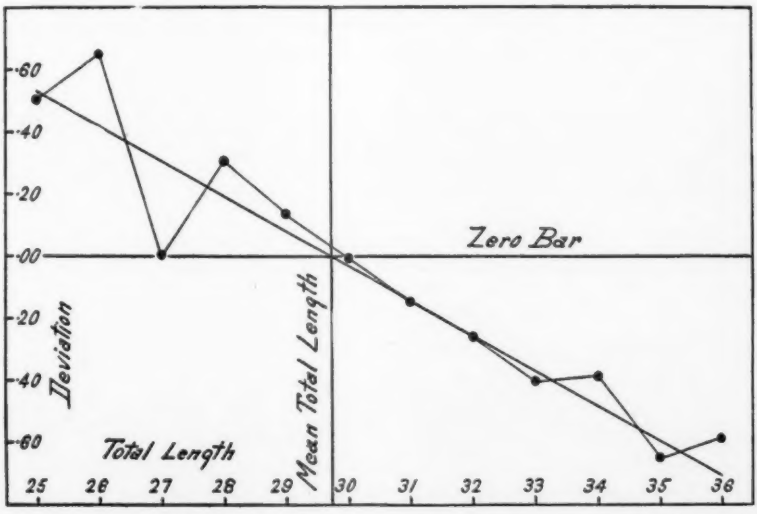

DIAGRAM 2.—Relationship of deviation of anterior length from its probable value to total length in Paramecium. Compare diagram 1.

Illustration 2. Absence of relationship between size of litter and sex in swine

PARKER and BULLARD (1913) have discussed the possible relationship between the size of the litter and sex in the contents of 1000 uteri of swine. From a simple percentage table they state that the relative numbers of males and females are "even in the extreme cases so nearly uniform that we may conclude with reasonable assurance that there is no intimate relation between sex and the size of the litters."

The correlations between the total numbers of pigs in the litter, l, and the number of males, m, and females, f, may be deduced from their data. They are

$$r_{lm} = .6833 \pm .0114$$
$$r_{lf} = .6875 \pm .0112$$

From these and the three coefficients of variation one may deduce

$$\text{For males} \quad r_{lz} = -.0177 \pm .0213$$
$$\text{For females} \quad r_{lz} = +.0177 \pm .0213$$

The correlation is sensibly zero, with regard to its probable error. This method of analysis therefore fully confirms the conclusion drawn by PARKER and BULLARD.

Illustration 3. Proportion of pistillate and hermaphrodite flowers in the inflorescence of the composite Homogyne

LUDWIG (1901) has given data for the correlation between the number of pistillate and the number of hermaphrodite flowers in the inflorescence of Homogyne. From his data we deduce

For hermaphrodite flowers, h,

$$\bar{h} = 31.8333, \ \sigma_h = 7.3981, \ V_h = 23.240$$

For pistillate flowers, p,

$$\bar{p} = 10.5370, \ \sigma_p = 2.6460, \ V_p = 25.112$$

For total flowers, f,

$$\bar{f} = 42.3704, \ \sigma_f = 8.7749, \ V_f = 20.7099$$

For hermaphrodite and pistillate flowers,

$$r_{hp} = .3899 \pm .0449$$

For total flowers and hermaphrodite flowers,

$$r_{fh} = .9607 \pm .0049, \ r_{fz_h} = .2429 \pm .0499$$

For total flowers and pistillate flowers,

$$r_{fp} = .6303 \pm .0339, \ r_{fz_p} = -.2429 \pm .0499$$

It follows, therefore, that in the larger heads the purely pistillate flowers are relatively less, and the hermaphrodite flowers relatively more, numerous.

Illustration 4. Fertility of capsules and viability of seed in carnation crosses

STUART (1912) has recorded the number of seeds obtained and the number germinated, planted into the field, and producing flowers in various carnation crosses. Our problem is to determine whether the seeds which come from capsules producing a large number of seeds are relatively more (or less) viable than those from capsules producing small numbers.

Using his two larger tables of data, tables 3 and 6,[3] and confining attention to the relationship between number of seeds per capsule, and the number which germinated, I find

For commercial × commercial, STUART's table 3, $N = 23$,

$$r_{sg} = .775 \pm .056, \qquad r_{sz} = -.072 \pm .141$$

For single flower × double flower, STUART's table 6, $N = 32$,

$$r_{sg} = .649 \pm .069, \qquad r_{sz} = -.118 \pm .118.$$

The signs are both negative, indicating a relatively higher failure to germinate among the seeds which are produced many in a capsule. With regard to their probable errors, the constants are untrustworthy. Because so few observations are available, no biological significance is attached to these two series, which serve merely as another illustration of the kind of problems to which the method may be applied.

Illustration 5. Relationship between the total number of pedicels and the number of abnormal pedicels in Spiraea Vanhouttei

In *Spiraea Vanhouttei* the pedicels of the umbel-like raceme normally produce but a single flower each. An abnormal condition in which one or more pedicels may bear a relatively large number of flowers is frequently observed (HARRIS 1917 d).

Let x be the total pedicels in an inflorescence and a the number which are abnormal. Then if abnormality be distributed purely at random among the pedicels one would expect material values of r_{xa}. The correlation r_{xz} meets our requirements since it shows whether inflorescences with a large number of rays have relatively more or fewer of their rays abnormal than those with a small number.

[3] In table 6 the cases in which the seeds are not normally developed are omitted.

During the last fifty years a great deal has been said about the influence of nutrition, vegetative vigor, etc., upon the development of anomalies. If a larger number of rays indicates greater vigor or better nutrition one might *a priori* expect larger inflorescences to have a proportionately higher number of branched rays, providing of course, that the classic theories are true.

The constants for a short series of data collected in 1906 were published in 1909. Since then a large number of determinations have been made on a general sample of inflorescences from a number of shrubs in 1909 and from three large individual shrubs in 1913.

In the latter series the data have been analyzed in two ways. First, the inflorescences which contain at least a single abnormal pedicel have been used as the basis of the correlations. These are designated as the abnormal inflorescences. Second, the normal inflorescences from the same plants have been included and counted as zero in the distribution of number of abnormal rays.

The results are:

For 1906[4] $r_{xa} = +.121 \pm .034$
$r_{xz} = -.071 \pm .034$

For 1909. Massed statistics. Inflorescences producing some abnormal pedicels ($N = 785$),

$r_{xa} = +.1542 \pm .0235$
$r_{xz} = -.0915 \pm .0239$

For 1909. Massed statistics. All 2040 inflorescences,

$r_{xa} = +.1584 \pm .0146$
$r_{xz} = +.0370 \pm .0149.$

For 1913. Individual plants. Inflorescences producing some abnormal pedicels,

Plant 1. $N = 747$ inflorescences.

$r_{xa} = +.0880 \pm .0244$
$r_{xz} = -.2846 \pm .0227$

Plant 2. $N = 641$ inflorescences.

$r_{xa} = +.1148 \pm .0263$
$r_{xz} = -.3855 \pm .0227$

[4] Since in the 1906 series only synanthies were observed, the total pedicels (including those with synanthies) were counted and the correlation is between the total pedicels and the pedicels with synanthies. In the 1909 material in which synanthies were rare, the correlation was determined for total pedicels and abnormal pedicels, abnormality being synanthy or any degree of branching.

Plant 3. $N = 548$ inflorescences.

$$r_{xa} = + .0941 \pm .0285$$
$$r_{xz} = - .2849 \pm .0265$$

For 1913. Individual plants. All inflorescences,

Plant 1. $N = 1135$ inflorescences.

$$r_{xa} = - .0067 \pm .0200$$
$$r_{xz} = - .2125 \pm .0191$$

Plant 2. $N = 975$ inflorescences.

$$r_{xa} = - .0821 \pm .0214$$
$$r_{xz} = - .3681 \pm .0187$$

Plant 3. $N = 912$ inflorescences.

$$r_{xa} = -.0360 \pm .0223$$
$$r_{xz} = - .2342 \pm .0211$$

For all the samples of inflorescences in which there is at least one abnormal pedicel the correlations between the total number of pedicels and the number of normal pedicels is positive in sign and perhaps statistically significant, but low in actual magnitude. Thus the number of abnormal pedicels increases on the average as the total number of pedicels per inflorescence becomes larger. The relationships are, however, very slight indeed.

For these five series the correlation between the total number of pedicels and the deviation of the abnormal pedicels from their probable value, is negative in sign. Thus the larger inflorescences have a relatively smaller proportion of abnormal pedicels than do those with a smaller total number of pedicels.

In the four series in which the wholly normal inflorescences are included, the correlations between total number of pedicels and number of abnormal pedicels is positive in 1909 but negative throughout and insignificant in magnitude in 1913. The three series from individual shrubs studied in 1913 show low but significantly negative correlations between the total number of pedicels per inflorescence and the deviation of the number of abnormal inflorescences from their probable value. The constant for the heterogeneous data of 1909 is positive but insignificant.

Taking the data altogether, there can be no reasonable doubt that the relative number of abnormal pedicels decreases as the total number of abnormal pedicels increases.

This is shown in diagram 3, which represents the regression of the deviation of the number of abnormal rays from their probable value on

the total number of rays in the series showing the lowest, and in one of these showing the highest, correlation.

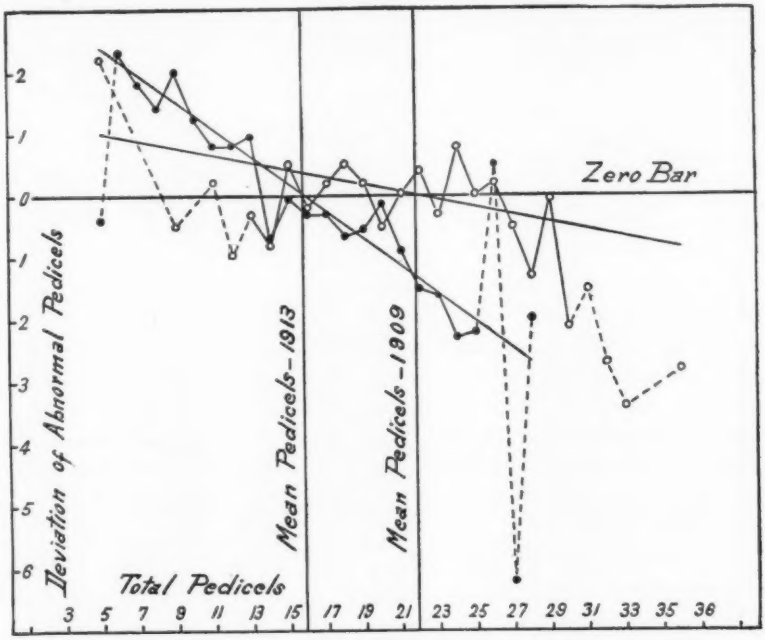

DIAGRAM 3.—Regression of the deviation of the number of abnormal pedicels from their probable value on the total number of pedicels in Spiraea.

The standard deviations are:

For 1909, $\sigma_z = 2.4691$.

For 1913, plant 1, $\sigma_z = 3.0527$.

The regression equations are:

For 1909, $z = 1.3186 - .0609\,x$.

For 1913, $z = 3.5036 - .2212\,x$.

Illustration 6. Interrelationship of cotyledons and primordial leaves in a race of Phaseolus vulgaris highly variable in seedling characters

The ontogeny of the flowering plant is usually divided into a primary period of dependent development and a sharply separated period of independent growth. The leaf homologs which are developed during the first period are usually of a very definite form and number, and frequently

highly differentiated from those developed after growth is resumed as an independent organism.

Those rare cases in which the number of leaf homologs which are laid down during the development of the seed is highly variable seem to offer especially favorable opportunities for the morphologist to learn something of the interrelationship of the two forms which may be assumed by homologous organs.

Suitable material for such work is furnished by a race of *Phaseolus vulgaris* (HARRIS 1916 b) having a modal number of four cotyledons and four primordial leaves, but highly variable in the number of both of these organs.

The correlations and regression equations are:[5]

Correlation between number of cotyledons and number of leaves:

Less mature series, $r = .1170 \pm .0320$.

$$c = 3.4759 + 0.0421\ l$$
$$l = 3.2919 + 0.3252\ c$$

More mature series, $r = .1568 \pm .0236$.

$$c = 3.5128 + 0.0499\ l$$
$$l = 3.0039 + 0.4922\ c$$

Combined series, $r = .1386 \pm .0193$.

$$c = 3.5030 + 0.0464\ l$$
$$l = 3.3565 + 0.4142\ c$$

The correlations between number of cotyledons and number of primordial leaves indicate slender interrelationships in this newly originated race between characters which are usually thought of as highly correlated.

Note from the equations that there is on the average a change of .3 to .5 of a leaf for each variation of one cotyledon, but that there is a change of only .04 of a cotyledon for a variation of one leaf.

The results for the combined series are represented graphically in diagram 4. Regression is for each character very nearly linear.

Correlation between total leaf homologs and number of cotyledons:[6]

Less mature series, $r = .4328 \pm .0264$.

$$c = 2.4595 + 0.1413\ h$$

[5] The plants were graded in a less mature and a more mature series as a precaution against recording as primordial leaves those really developed subsequently to the maturation of the seed.

[6] The following correlations are published here for the first time.

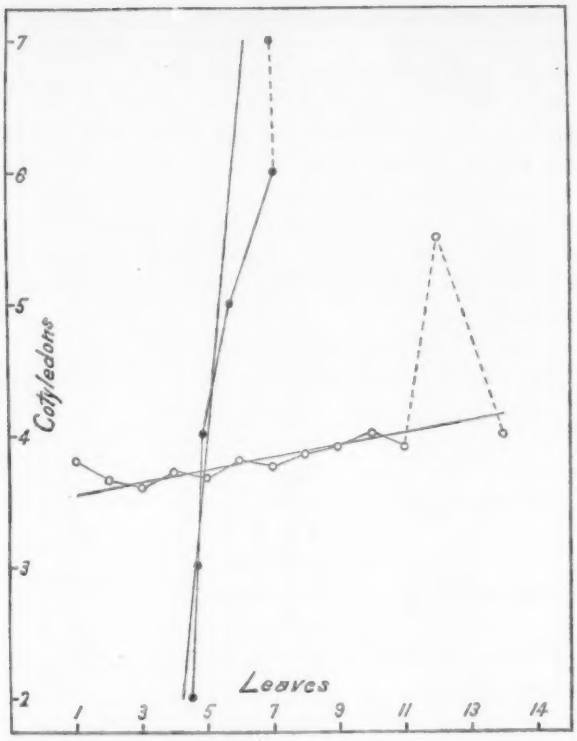

DIAGRAM 4.—Relationship between number of cotyledons and number of primordial leaves in a tetracotyledonous race of *Phaseolus vulgaris*.

More mature series, $r = .4337 \pm .0197$.

$$c = 2.6704 + 0.1260\,h$$

Combined series, $r = .4312 \pm .0084$.

$$c = 2.5954 + 0.1314\,h$$

The straight line showing the regression of number of cotyledons, c, on total leaf homologs, h, is shown with the empirical means in diagram 5.

Correlation between total leaf homologs and number of leaves:

Less mature series, $r = .9459 \pm .0034$.

$$l = -2.4595 + 0.8586\,h$$

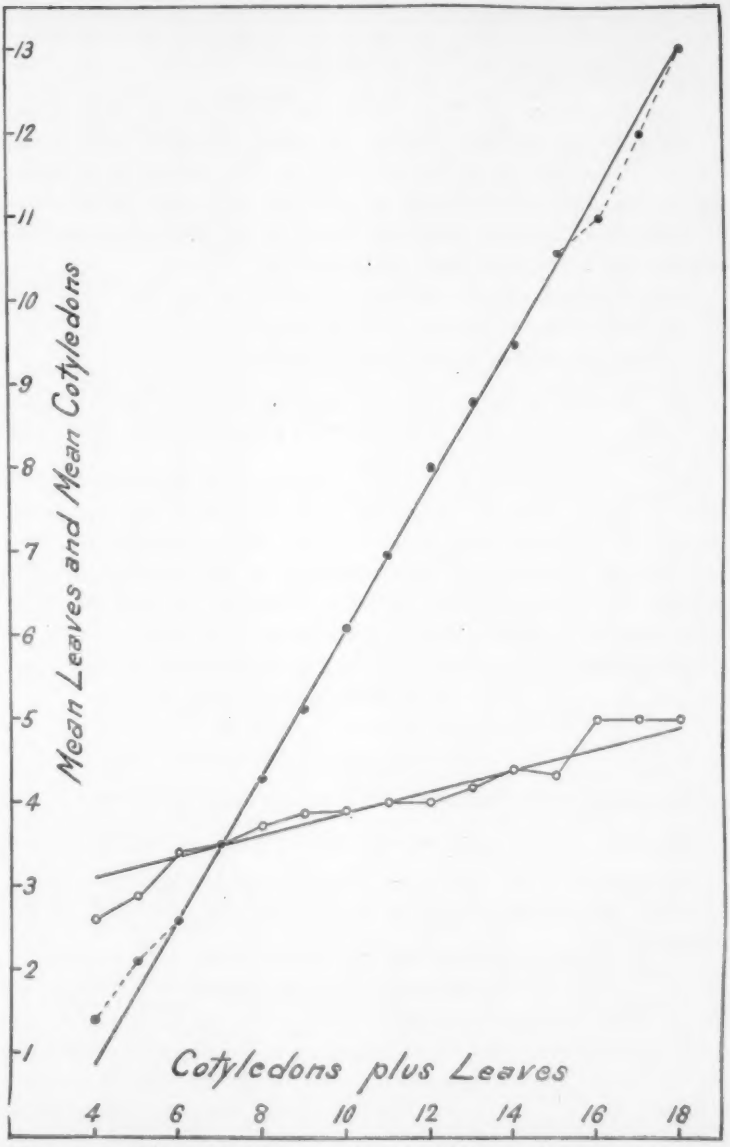

DIAGRAM 5.—Relationship between number of cotyledons and number of leaves and total number of leaf homologs (cotyledons + leaves) in Phaseolus.

More mature series, $r = .9579 \pm .0020$
$l = -2.6704 + 0.8740\ h$
Combined series, $r = .9533 \pm .0018$.
$l = -2.5953 + 0.8685\ h$

Diagram 5 gives the regression of number of leaves on the number of leaf homologs (cotyledons + leaves). The solid dots representing the empirical mean leaf number lie practically on the theoretical line.

Correlation between total leaf homologs and the deviation of the number of leaves from their probable value:

Less mature series, $r = .6936 \pm .0226$.
More mature series, $r = .7642 \pm .0185$.
Combined series, $r = .7378 \pm .0089$.

The correlations between total leaf homologs and the deviations of the cotyledons from their probable value are numerically identical with the foregoing but negative in sign.

The results show a high degree of consistency of the two series. These final constants show that when the total number of leaf homologs increases, the variation is due to a far greater extent to the laying down of a greater number of primordial leaves than to the formation of a larger number of cotyledons. The regression equations for both the deviation of the number of leaves from their probable value (z_l) and the deviation of the number of cotyledons from their probable value (z_c) are given by

$$\sigma_z = .874557$$
$$z_c = 2.5954 - 0.3007\ h$$
$$z_l = -2.5954 + 0.3007\ h$$

and represented in diagram 6. The results are clearly linear.

Illustration 7. Change in proportion of parts in developing trout

JENKINSON (1912) has given data for total length and length of head for three growth stages in the American rainbow trout. His constants are:

	Total length, l CV	Head length, h CV	Total length and head length r_{lh}
Stage 1	13.11	19.68	.945
Stage 3	5.11	6.57	.729
Stage 5	7.74	6.55	.848

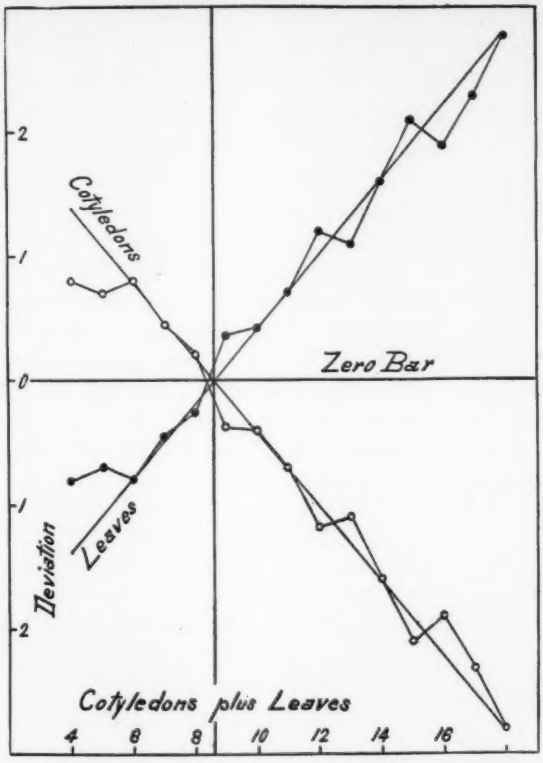

DIAGRAM 6.—Regression of the deviation of the number of cotyledons and of the number of leaves from their probable numbers on the total number of leaf homologs in Phaseolus. Note that the theoretical lines and the empirical means are identical but opposite in sign.

Whence we deduce:

Stage 1, $r_{lz} = + .649 \pm .027$.
Stage 3, $r_{lz} = - .071 \pm .047$.
Stage 5, $r_{lz} = - .534 \pm .038$.

These results bring out especially well the analytical value of the method here proposed. The correlations between total length and head length are *roughly* the same in the three stages of development. The correlation between total length and deviation of head length is at first strongly positive, then sensibly zero in the third stage and finally becomes negative and of the order — .5 in the fifth stage.

Thus while in each stage the larger individuals have the larger heads, the relationships between the total length of the body and the proportional length of the head changes greatly during development.

Illustration 8. Relationship between total solids and sucrose content in the juice of sugar beets

Sucrose content of the juice of the sugar beet has been one of the classic examples of variation in text-books on genetics. Nevertheless our knowledge of the problems are, as indicated by a recent review (HARRIS 1917 b), far from complete. Relatively little is known, for example, of the correlation between total solids and sucrose content. Yet this problem is not merely of physiological and genetical interest but of very great practical importance as well, since the coefficient of purity of the juice is an important factor in sugar manufacture.

Table 4 shows the correlation between total solids and sucrose content in 475 Nevada sugar beets. Nominally this series is composed of a number of commercial varieties, but since it has been shown elsewhere (HARRIS and GORTNER 1913) that the correlation between weight and composition of juice is not greatly influenced by the combination of these nominal varieties, such heterogeneity as exists probably does not influence materially the correlations to be deduced. For purposes of comparison the constants for a series of 61 determinations from Washington are added.

The Nevada series gives:

For total solids,

$$\text{Mean} = 18.021,\ \sigma = 3.7224,\ \text{CV} = 20.656$$

For sucrose,

$$\text{Mean} = 14.989,\ \sigma = 4.0727,\ \text{CV} = 27.171$$

For total solids and sucrose,

$$r = .882 \pm .007$$

For total solids and deviation of sucrose from its probable value,

$$r_{ss} = + .251 \pm .029$$

For the Washington series the results are:

For total solids,

$$\text{Mean} = 16.445,\ \sigma = 1.5896,\ \text{CV} = 9.666$$

For sucrose,

$$\text{Mean} = 11.838,\ \sigma = 1.9098,\ \text{CV} = 16.133$$

For total solids and sucrose,

$$r = .866 \pm .022$$

TABLE 4

Total solids and sucrose content in sugar beets.

Total solids \ Sucrose content	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Totals
6	—	—	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
9	—	1	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
10	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
11	—	—	—	—	—	1	4	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7
12	—	—	—	1	—	3	3	5	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	14
13	—	—	—	—	—	—	1	4	3	10	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	21
14	—	—	—	—	1	1	1	3	8	6	8	6	1	—	—	—	1	—	—	—	—	—	—	—	—	36
15	—	—	—	1	—	—	1	—	7	3	15	9	5	—	—	—	—	—	—	—	—	—	—	—	—	41
16	—	—	—	—	—	1	1	1	—	1	6	9	13	4	1	—	—	—	—	—	—	—	—	—	—	37
17	—	1	—	—	—	—	—	1	—	1	1	5	9	16	5	1	—	—	—	—	—	—	—	—	—	40
18	—	—	—	—	—	—	—	1	—	—	2	3	7	10	20	9	1	—	—	—	—	—	—	—	—	53
19	—	—	—	—	1	—	—	1	—	—	2	1	3	4	7	17	6	—	—	—	—	—	—	—	—	42
20	—	—	—	—	—	—	—	—	—	1	1	1	—	1	4	18	28	3	—	1	—	—	—	—	—	58
21	—	—	—	—	—	—	—	—	—	—	—	2	—	4	2	2	8	5	10	1	—	—	—	—	—	34
22	—	—	—	—	—	—	—	—	—	—	—	1	—	3	1	3	5	9	5	3	1	—	—	—	—	31
23	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	2	1	1	5	8	2	—	—	—	—	20
24	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	3	9	5	2	—	—	—	22
25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	3	—	—	—	—	4
26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	2	—	—	—	4
27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	—	—	2
34	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
Totals	1	2	2	4	2	6	13	18	21	22	37	38	38	42	40	53	51	19	23	24	13	4	1	—	1	475

For total solids and deviation of sucrose from its probable value,

$$r_{sz} = .471 \pm .067$$

Thus in both cases the correlation between total solids and sucrose is moderately high, as is necessarily the case because of the fact that total solids are to a considerable extent made up of sucrose.

Quantity of sucrose is in both cases more variable than the total amount of solids. The correlation between total solids and the relative amount of sucrose is in both cases positive and of a substantial order of magnitude. Beet juice with higher total solids contains both absolutely more and relatively more sugar than that of beets with low total solid content.

Another way of approaching the problem is to calculate the correlations between total solids and the coefficient of purity, which is often determined.

Nevada series,

$$r_{sp} = .340 \pm .027$$

Washington series,

$$r_{sp} = .529 \pm .062$$

These values are also positive and substantial, but their interpretation involves difficulties due to spurious correlation.

Illustration 9. Relationship between total number of spikelets and number of sterile spikelets in wheat

GRANTHAM and GROFF (1916) have given data for the correlation between the total number of spikelets and the number of sterile spikelets at the base of the head in varieties of wheat. Let t = total spikelets and s = sterile spikelets. Then from their two tables I find, without applying SHEPPARD'S correction,

Bearded varieties,

$$r_{ts} = .635 \pm .039,\ r_{ts_t} = +.164 \pm .063$$

Beardless varieties,

$$r_{ts} = .542 \pm .054,\ r_{ts_t} = +.232 \pm .072$$

For both groups of varieties there is a positive correlation between total spikelets and sterile spikelets and between total spikelets and the deviation of the number of sterile spikelets from their probable value. Sterility is, therefore, not merely absolutely but relatively more frequent in varieties with larger numbers of spikelets.

Illustration 10. Viability of dominants and recessives in F_2 generation of Mendelian hybrids

F_1 D(R) × D(R), according to Mendelian theory, gives in F_2 a population with somas 3 D : R. Let there be N individual families in this population and the number of individuals per family be variable. This variation may be due solely to fluctuations in the number of zygotes produced, or it may be in part attributable to the failure of some of the zygotes to reach maturity. This failure may be random or differential with respect to the alternative characters involved. If differential, one might expect a correlation between the actual number of individuals per family and the deviation from the probable number of either of the alternative types of individuals which it contains.

In current Mendelian literature discrepancies between observed and theoretical ratios are often explained as due to selective fertilization or to a lower viability of particular zygotes.

If the latter explanation be correct r_{lz}, the correlation between the number in the litter and the deviation from their probable frequency of the number which shows a particular character, should have a statistically significant value. As an illustration of the kind of data to which this test may be applied, I take YULE's (1914) table of DARBISHIRE's (1904) results for albinos in mice families. If the fertilized ovum which is to produce an albino be less capable of development than that which is to develop into a normal individual r_{lz} should take a substantial positive value, i.e., the relative numbers of albino young should be larger in the large litters, since the small litters are assumed to be small *in part* because albino-producing fertilized ova are supposed to be less viable.

The actual results are:

$$\begin{aligned} \bar{l} &= 4.5868 & \bar{a} &= 1.1322 \\ \sigma_l &= 1.7518 & \sigma_a &= 1.0752 \\ V_l &= 38.193 & V_a &= 94.963 \end{aligned}$$

$$r_{la} = .3756 \pm .0527$$
$$r_{lz} = -.0287 \pm .0612$$

where l = number in litter, a = number of albinos in litter, and z = deviation of number of albinos from their probable value.

The constant of critical significance, r_{lz}, is sensibly zero, with due regard to its probable error. Hence there is no evidence of a greater intra-uterine mortality of albino zygotes.

RECAPITULATION

In many instances the biologist has to consider the relationship between a measurement and some of its logical subdivisions or components. The first has been called an independent and the second, which is always some fraction of the first, a dependent variable.

For the analysis of such relationships two coefficients are required, the correlation between the variable and the dependent variable and the correlation between the variable and the deviation of the dependent variable from its probable value on the assumption that the relative magnitude of the dependent variable is independent of the magnitude of the variable.

This paper gives (*a*) the supplementary formulae which are required in certain cases in which the correlation between a variable and the deviation of a dependent variable from its probable value is to be computed, and (*b*) a series of illustrations of the applicability of this coefficient, drawn from a wide range of biological phenomena.

The method has been most extensively applied to the problems of the physiology of seed production in plants. The relationship of the number of ovaries which develop to maturity to the total number of ovaries formed, and the relationship of the number of seeds which mature to the total number of ovules laid down, have been determined in a considerable number of plant forms. That it may be useful in the study of the relationship of seed viability to the number of seed formed is shown by analysis of meagre data for carnation crosses.

The formulae have been applied to the problem of sex by an investigation of the relative proportion of macrosporophylls and microsporophylls in data for Arisarum, Ficaria and Homogyne, and for the relative numbers of males and females in litters of swine.

Under certain conditions the method may be of use in testing the assumption of the existence of a differential viability in Mendelian dominants and recessives.

The usefulness of the method in morphology has been illustrated by its application to the problem of the relationship between total length and anterior length in Paramecium, to that of the relative size of the head in developing trout, to that of the relative frequency of abnormal pedicels in the inflorescence of Spiraea, and to that of the relationship of the numbers of cotyledons and primordial leaves to the total number of leaf homologs in a highly variable race of Phaseolus. Such studies have not merely an independent morphological value, but have their bearing

upon such biological theories as DRIESCH's annunciation that in a differentiated system the proportionality of the parts is absolutely independent of the size of the system.

In dealing with materials of economic importance, the method has been applied to the problem of the relative proportion of sterile spikelets in varieties of wheat with varying numbers of total spikelets per head, to the relationship between total solids and sucrose content in the juice of sugar beets, and has been found of the greatest value in analyzing the relationship between the egg production of the individual months and that of the entire year in the domestic fowl.

These illustrations are perhaps sufficient to show that the coefficient has great analytical value, and should have the widest usefulness.

Finally it must be understood that the conclusions drawn from the illustrations given in this paper are not in all cases to be extended beyond the specific series of data to which the formulae have been applied. The data employed are, *in some instances*, intended to illustrate the type of biological problem to which the formulae may be applied when more extensive data are available. No attempt has been made to discuss in detail the biological significance of results of so diverse kinds of illustrations. Such discussion must be left to the specialist in the particular field in which the observations fall.

LITERATURE CITED

DARBISHIRE, A. D., 1904 On the result of crossing Japanese waltzing with albino mice. Biometrika **3**: 1-51.

GRANTHAM, A. E., and GROFF, F., 1916 Occurrence of sterile spikelets in wheat. Jour. Agr. Res. **6**: 235-250.

HARRIS, J. ARTHUR, 1909a The correlation between a variable and the deviation of a dependent variable from its probable value. Biometrika **6**: 436-443.

1909b Correlation in the inflorescence of *Celastrus scandens*. Ann. Rep. Mo. Bot. Gard. **20**: 116-122.

1910a A quantitative study of the morphology of the blood root, *Sanguinaria canadense*. Biometrika **7**: 305-351.

1910b The arithmetic of the product moment method of calculating the coefficient of correlation. Amer. Nat. **44**: 693-699.

1912 Biometric data on the inflorescence and fruit of *Crinum longifolium*. Ann. Rep. Mo. Bot. Gard. **23**: 75-99.

1913 On the relationship between the number of ovules formed and the capacity of the ovary for maturing its ovules into seeds. Bull. Torr. Bot. Club **40**: 447-455.

1914a On the relationship between the number of ovules formed and the number of seeds developing in Cercis. Bull. Torr. Bot. Club. **41**: 243-256.

1914b Further observations on the relationship between the number of ovules formed and the number of seeds developing in Cercis. Bull. Torr. Bot. Club **41**: 533-549.

HARRIS, J. ARTHUR, 1916 a On the distribution and correlation of the sexes (staminate and pistillate flowers) in the inflorescence of the aroids *Arisarum vulgare* and *Arisarum proboscidium*. Bull. Torr. Bot. Club **42**: 663-673.

1916 b A tetracotyledonous race of *Phaseolus vulgaris*. Mem. N. Y. Bot. Gard. **6**: 229-244, *figs. 1-3*.

1917 a Supplementary determinations of the relationship between the number of ovules per pod and fertility in Phaseolus. Genetics **2**: 282-290.

1917 b Biometric studies on the somatic and genetic physiology of the sugar beet. Amer. Nat. **51**: 507-512.

1917 c The correlation between a component, and between the sum of two or more components, and the sum of the remaining components of a variable. Quart. Publ. Amer. Stat. Ass. **15**: 854-859. *pls. 30, 31*.

1918 a The interrelationship of the number of stamens and pistils in the flowers of Ficaria. Biol. Bull. **34**: 7-17, *figs. 1-5*.

1918 b On the distribution of abnormalities in the inflorescence of *Spiraea Vanhouttei*. Amer. Jour. Bot. **4**: 624-636, *diagrams 1-4, pls. 30, 31*.

HARRIS, J. ARTHUR, and BLAKESLEE, A. F., with the coöperation of KIRKPATRICK, WM. F., 1917 Inter-periodic correlation in the egg production of the domestic fowl. Proc. Nat. Acad. Sci. **3**: 565-569, *figs. 1, 2*.

HARRIS, J. ARTHUR, and GORTNER, R. A., 1913 On the relationship between the weight of the sugar beet and the composition of its juice. Jour. Ind. Eng. Chem. **5**: 192-195.

1918 The correlation between egg production during the various periods of the year in the domestic fowl. Genetics **3**: 27-72, *figs. 1-11*.

JENKINSON, J. W., 1912 Growth, variability and correlation in young trout. Biometrika **8**: 444-455.

JENNINGS, H. S., 1911 Assortative mating, variability and inheritance of size in the conjugation of Paramecium. Jour. Exp. Zoöl. **11**: 1-134.

LUDWIG, F., 1901 Variationsstatistische Probleme und Materialien. Biometrika **1**: 11-29.

PARKER, G. H., and BULLARD, C., 1913 On the size of litters and the numbers of nipples in swine. Proc. Amer. Acad. Arts and Sci. **49**: 399-426.

PEARL, R., 1909 The frequency constants of a variable $z = f(x_1, x_2)$. Biometrika **6**: 437-438.

STUART, W., 1912 Mendelian inheritance in the carnation. Bull. Vt. Agr. Exp. Sta. 163.

YULE, G. U., 1914 Fluctuations of sampling in Mendelian ratios. Proc. Cambridge Phil. Soc. **17**: 425-432.

STUDIES ON SELF-STERILITY II. POLLEN-TUBE GROWTH

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TABLE OF CONTENTS

PAGE
INTRODUCTION 353
Historical 354
Pollen-tube growth in artificial media 356
Attempts to self-pollinate mutilated pistols 358
Pollen-tube growth in the pistil 359
SUMMARY AND DISCUSSION 362
LITERATURE CITED 365

INTRODUCTION

In the first paper of this series (EAST and PARK 1917), various pedigree culture experiments involving the behavior of self-sterile Nicotianas when crossed or selfed were described in considerable detail. Among the points there established are the following:

1. The four species *N. Forgetiana, N. alata, N. glutinosa* and *N. angustifolia* are truly self-sterile, but the character is affected by attendant conditions in rather a peculiar way. The plants are completely self-sterile during the active part of the flowering season, but toward the close of this period, especially under adverse conditions, some self-fertility may be shown. *N. alata* and *N. glutinosa* are influenced thus more markedly than *N. Forgetiana* and *N. angustifolia*. As a direct corollary of this fact it follows that self-sterility is only a physiological impediment to self-fertilization.

2. Cross-sterility, apparently of the same nature as self-sterility, exists.

3. Cross-sterility exhibits a regularity in its behavior such that if plant A is sterile with plant B and with plant C, it may be predicted that plant B will be sterile with plant C. On the basis of this cross-sterility the plants within a family or even within a series of families may be divided into a comparatively small number of groups in which each member of a group is sterile with every other member of that group and fertile with every member of every other group.

4. Excluding the pseudo-fertility sometimes manifested during the wane of the flowering season and true sterility which is due to non-functional gametes, there is no fluctuation in fertility in compatible combinations. Incompatible combinations produce no seed, compatible combinations are fully fertile.

5. Reciprocal crosses always behave in the same manner; from which it follows that the effective hereditary factors controlling compatibility partake of the nature of the sporophyte rather than the gametophyte generation.

The immediate cause of these peculiar manifestations is the varied rate of pollen-tube growth characteristic of compatible and incompatible combinations. It is with these phenomena the present paper is concerned.

HISTORICAL

Numerous researches on pollen-tube growth have been made during the last three or four decades, but owing to conflicting results, comparatively few contributions to knowledge have resulted.

VAN TIEGHEM (1869) appears to have been the first investigator to demonstrate pollen germination. He also showed that certain pollen grains contain enzymes which invert cane sugar. This conclusion was corroborated by STRASBURGER in 1886, who found also that starch could be transformed into sugar, an indication of diastase. GREEN (1894) succeeded in isolating both of these substances, but could not demonstrate the presence of cytolase, an enzyme he believed must be concerned in pollen-tube growth from the fact that growth is intercellular. GREEN concluded that the reserve foods of the pollen grain are starch, dextrin, cane sugar, maltose and glucose, the style containing the same substances with the exception of dextrin. These conclusions, however, can hardly be accepted without question.

Several authors have assumed that the pollen grains of certain species have developed specific chemical requirements for germination, thus accounting for their failure to obtain growth on artificial media; but this can hardly be true since pollen grains will so often germinate on stigmas of plants belonging to a different species or even to another genus, to say nothing of the successes that have been obtained on artificial media varying from comparatively pure water to the pure (?) agar agar of the trade. (Cf. STRASBURGER 1886; RITTINGHAUS 1887; MOLISCH 1893; LIDFORSS 1896, 1899; JOST 1905, 1907; TOKUGAWA 1914). Questioning this conclusion, however, does not imply a denial that in certain cases a preference may have been developed for particu-

lar substances. In other words, pollen grains may germinate and grow on a variety of media, but the rate of growth may be much greater in some cases than in others. No other conclusion seems to interpret appropriately the results of MOLISCH (1893), MIYOSHI (1894), LIDFORSS (1896), JOST (1907) and ourselves, even though we agree with STOUT (1916) that experiments on artificial media are rather unsatisfactory.

MOLISCH (1893) believed he had demonstrated both anaerotropism and chemotropism for acids and for secretions of the gynæcium, especially those of the stigma. He did not assume chemotropism to be a general phenomenon, for there are pollen tubes (e.g. *Orobus vernus*) which neither shun the air nor are attracted by the stigmas; nevertheless he did feel that chemotropism plays an important rôle in the passage of the pollen tube to the egg cell. MOLISCH unquestionably made a serious attack on the problem, for he investigated over 100 species.

LIDFORSS (1899) confirmed MOLISCH's observation that the pollen tubes of *Narcissus tazetta* grow toward their own stigmas in a gelatin medium, but his endeavors to imitate the effect of the stigmas with various sugars, organic acids, amides and tannins were without success. On the other hand, he did succeed in attracting the pollen tubes by the use of pieces of onion bulb and by granules of a commercial preparation of diastase. He believed that this success was due to certain proteins, since the diastase still attracted the tubes after the ferment was killed by heat, and since egg albumen washed free of mineral salts exerted the same effect. Casein and "taka" diastase were without effect, but this was thought to be dependent upon the presence in them of mineral salts.

He states that pollen tubes of *Fritillaria imperialis* are more sensitive to salts than Narcissus. The former were killed by the same diastase preparation that had attracted the Narcissus tubes. On the other hand, dialyzed egg albumen exerted a strong attraction for the Fritillaria pollen tubes. Numerous other experiments were carried out in which the effect of proteins on various species of Choripetalæ was tested. No tendency to attract was discovered, but this he believed to be due to their great sensitiveness to small amounts of salts.

MIYOSHI (1894) found that the stigma and style of many angiosperms contained reducing sugars. Chemotropic effects on their pollen tubes were obtained by the use of several different sugars and dextrins in a gelatin medium. Meat extract, asparagin, glycerine and gum arabic had no effect, and alcohol and certain salts excited more or less repulsion.

Perhaps the most general conclusion of MIYOSHI was that pollen

tubes could be turned from one solution to another if the concentration of the second be increased as demanded by WEBER's law.

These experiments, as well as the later ones along the same lines made by MARTIN (1913), TOKUGAWA (1914), ANDRONESCU (1915), ADAMS (1916) and others, must be accepted with some reservation. There is certainly a probability that pollen tubes show chemotropism, but it must be admitted, as STOUT (1916) maintains, that the amount of pollen-tube growth observed in artificial media is small, probably never over 1.5 mm. This being the case, one is likely to be over-influenced by working hypotheses, and to conclude in favor of chemotropism without due evidence. At the same time, these investigators must be thanked for having given us a general idea, though perhaps somewhat superficial, of the physiology of the pollen tube.

The only data on pollen-tube growth bearing directly on the problem of self-sterility are those of JOST (1907) and of CORRENS (1912). They found that when a self-sterile plant is pollinated with its own pollen, the tubes are emitted freely but grow extremely slowly. Since a cross-pollination on the same plant results in rapidly growing tubes, the hypothesis was advanced, somewhat differently by each, that special substances in each plant inhibit the growth of pollen tubes from pollen of that plant.

POLLEN-TUBE GROWTH IN ARTIFICIAL MEDIA

Our own experiments on pollen physiology through the use of artificial media were made on *N. angustifolia* and *N. Forgetiana*. The medium usually employed was 2 percent agar agar to which various nutrients were added, although pure agar was used successfully. As nutrients different percentages (1-20) of cane sugar, glucose, levulose and sodium malate were used. The tubes grew well on all of these media, the best development being obtained on 2 percent agar plus 20 percent cane sugar. The maximum growth in this medium was about .6 mm. This length of pollen-tube is almost negligible when compared with the 3 cm to 7 cm necessary for the sperm nucleus to reach the ovule. Nevertheless, there seems to be no question but that there is a true germination and a real growth on artificial media. If the pollen grains are perfect morphologically, that is if no true pollen sterility is present, pollen tubes are formed in nearly every case. Owing to the comparatively short length to which they grow, one is hardly justified in plotting a growth curve, but there is no doubt but that the rate of growth from germination onward is either progressively slower, or that it starts

slowly, reaches a maximum in from 12 to 24 hours and then falls off. This fact should not be overlooked, as it is not what occurs when a natural compatible mating is made, and shows clearly the great difference between pollen-tube growth in a natural and in an artificial medium.

In over 100 experiments of this type a high percentage of pollen germination (70-80 percent) was obtained, and the pollen tubes grew well no matter what medium was used. The tubes were emitted in all directions, but in general the growth of an individual tube was in one direction, though there was considerable variation from a straight line.

Since the experiments of MOLISCH, LIDFORSS, MIYOSHI and others all indicated that pollen tubes are attracted toward certain substances, it was thought that possibly the same phenomenon might occur if portions of the gynæcium of flowers of the same species were placed in the media. Many experiments were tried therefore in which parts of stigmas, styles and ovaries (both crushed and uncrushed) or of their extracts were placed in the media and pollen scattered near them at various distances (.5 to 3 cm). In some cases gynæcium parts from the same self-sterile plant which furnished the pollen were used, in other instances the gynæcium parts came from one plant and the pollen from a plant cross-sterile with the first. The tendency of the pollen tubes in these tests was compared with that of pollen from the same plants when placed near gynæcium parts of plants with which the pollen was known to be compatible. We were not able to discover any difference in the behavior of the pollen tubes in these trials. Occasionally, perhaps in 10 percent of the cases, the pollen tubes seemed to be attracted by the gynæcium parts, but the percentage was about the same in all cases. If there was really any attraction at all, which is doubtful, it was no greater between plant parts from plants known to be capable of effecting mutual cross-fertilization than it was from plant parts taken from plants which were cross-sterile together, or even when taken from the same plant. Notwithstanding the fact that there was no decided turning of the tubes toward any object or substance placed in the medium, there was some evidence that the presence of gynæcium parts promoted growth. On a number of occasions data were secured such as are shown in table 1.

The evidence of stimulation from the presence of ovules and more particularly of stigmas is unmistakable but whether the presence of "compatible" stigmas or ovules shows an additional stimulation over that due to "incompatible" stigmas and ovules is doubtful.

TABLE 1

Growth of pollen tubes in a 2 percent agar medium in VAN TIEGHEM *cells. Plants A and B are both self-sterile, but are cross-fertile.*

Exp.	Materials used	Ave. length tubes in μ
1	Pollen A + ovary B crushed	24
	Pollen A + stigma B crushed	24
	Pollen B + ovary A crushed	18
	Pollen B + ovary B crushed	8
	Pollen B + stigma B crushed	20
	Pollen A only	8
	Pollen B only	14
2	Pollen A + stigma A	120
	Pollen A + stigma B	170
	Pollen A	40
	Pollen B + stigma B	100
	Pollen B + ovary B	88
	Pollen B + ovary A	100
	Pollen B	50
3	Pollen B + stigma B	60
	Pollen B + stigma A	100
	Pollen B + ovary B	30
	Pollen B + ovary A	50
	Pollen B only	30
	Pollen A only	10

Note: Data taken after 24 hours in exp. 1, after 48 hours in exp. 2, and after 24 hours in exp. 3.

These results do not corroborate the work of the earlier writers mentioned above, but neither do they prove there is no such thing as pollen-tube chemotropism. They indicate some sort of nutritive value or stimulative effect of substances contained in gynæcium parts, but there is no evidence that gynæcium parts are more nutritive than other plant parts nor that "compatible" plant parts are better than "incompatible" plant parts. Experiments of this kind are unsatisfactory. They may not be useless, but it seems improbable that any notable increase in knowledge will be obtained by their use until the technique is so improved that the growth curve in artificial media compares favorably with the natural growth curve.

ATTEMPTS TO SELF-POLLINATE MUTILATED PISTILS

In the early part of our work numerous attempts were made to obtain selfed seed on self-sterile plants by endeavoring to force pollen

tubes to grow in shortened styles. The experiments were of two types. Various methods of obtaining temporary unions between stigma and style after excising portions of the latter were tried by means of wax and glass envelopes. All of these attempts were unsuccessful. Pollinations of decapitated pistils were also made using stigmatic fluid and various sugars as germination media. In two cases, seed was obtained where stigmatic fluid was used. The matter is merely mentioned to show the possibility of developing a successful technique. The experiments were discontinued as soon as it had been proved by end-season self-pollinations that self-fertilization of self-sterile plants is possible, and that for this reason self-sterility is no true impediment to the fusion of an egg with a sperm nucleus which is the product of the same plant.

POLLEN-TUBE GROWTH IN THE PISTIL

The most gratifying experiments along these lines were those concerned with the rate of growth of pollen tubes in the pistils of self-pollinated and of cross-pollinated plants. Studies were made on pistils of *N. Forgetiana, N. alata* and *N. angustifolia*. The technique consisted of making series of self-pollinations and of cross-pollinations, both compatible and incompatible, on a single plant, collecting the pistils thus treated on successive days, and fixing, dehydrating, imbedding and sectioning them in the usual manner. Longitudinal sections about 10μ thick were used. Triple staining with safranin, gentian violet and orange G gave the best results, although safranin alone was almost as satisfactory.

About 400 slides each containing from 10 to 30 serial sections were prepared in this manner.

The germination of the pollen was found to be just as high in the selfed as in the crossed pistils, and the number of pollen tubes in a single pistil was very large. By actual count it was determined that single pistils may contain from 1200 to 2000 tubes, a number sufficient to fertilize from 4 to 6 times the number of ovules in the ovary.

The distribution of pollen tubes in the selfed pistils was always minus skew as is shown by the following sample frequencies.

Distance from stigma mm	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5
1 section after 6 days	1	43	30	30	20	13	7	7	5	2	1	1	
1 section after 7 days				4	20	24	18	9	7	2	2	1	

The large number of short pollen tubes and the few greatly in the lead raise the important question: Do some pollen tubes grow faster

than others because they have different genetic constitutions? Should this be so, selective fertilization would result. We have good evidence, however, that this is not the case.[1] The difference in length of the various pollen tubes is probably due largely to variation in the time of germination. Those pollen grains which are in intimate contact with the moist stigma absorb its secretions and put forth their tubes more quickly than do those less favorably situated. If, therefore, a flower be pollinated at a particular time, one may accept the mode of the frequency distribution of the pollen tubes within the pistil as a proper measure of pollen-tube growth for a given period.

A considerable amount of data of this kind has been collected for the purpose of comparing the rate of pollen-tube growth in selfings, and in cross-fertile and cross-sterile matings. Figures 1, 2 and 3 are graphs made from a random sample of the results secured. Each point represents the estimated modal length of the pollen tubes in a pistil collected at the indicated length of time after pollination. Ordinates to the various broken lines at the top of the figure represent the total length of the pistil of the respective plants. Figures 1 and 2 are from studies on *Nicotiana Forgetiana*. The rate of growth of selfed pistils in figure 1 and of the composite of selfed pistils in figure 2 are typical of selfed plants during the active part of the flowering season. The growth curve is practically a straight line. The pollen tubes grow well; as far as one may judge visually there is no difference between them and tubes from cross-pollinations. But they grow so slowly that even after the extraordinary flower life attained on plant 3 where a 14-day point is recorded, the pollen tubes are only half way to the ovary.

In crossed pistils, on the other hand, if the mating is compatible, growth curves are produced which are of a very different character. The pollen tubes start to grow at about the same rate as in selfed pistils, but the speed continually increases until fertilization ensues,—usually after from 3 to 5 days. The curve simulates that of an autocatalytic reaction.

The points plotted for selfings on plants 6 and 7, shown in figure 2 produce curves intermediate between the true "self" curves and the "cross" curves. This is the type of change that is brought about late in the flowering season and which when carried to an extreme produces pseudo self-fertility.

Figure 3 represents curves similar to those just described, but com-

[1] For a preliminary report on this subject see Jour. Heredity 8: 382, 1917. Further details will be published later.

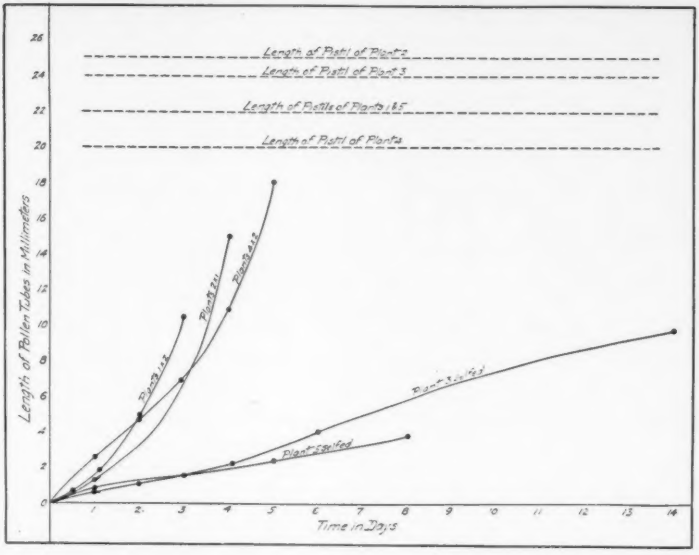
Length of Pistil of Plant 2
Length of Pistil of Plant 3
Length of Pistils of Plants 1 & 5
Length of Pistil of Plant 4
Length of Pollen Tubes in Millimeters
Time in Days

FIGURE 1.—Growth curves of pollen tubes from selfed and from crossed pistils of plants of *Nicotiana Forgetiana*.

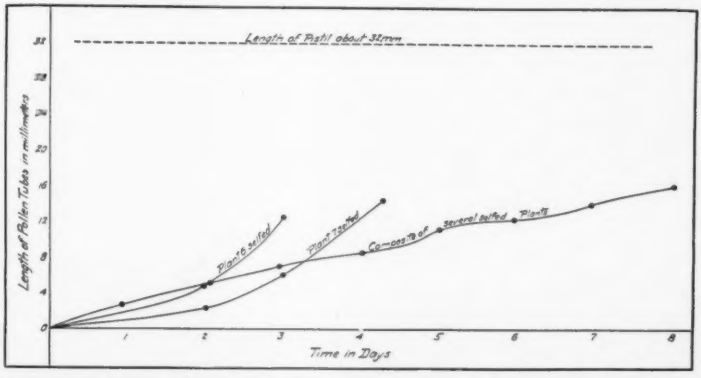

FIGURE 2.—A composite growth curve of pollen tubes from normal selfed pistils of two plants of *Nicotiana Forgetiana*. End-season growth curves from selfed pistils of plants of the same species.

piled from data upon *N. angustifolia*. The selfings produce straight lines and the compatible crosses produce curves in which pollen-tube

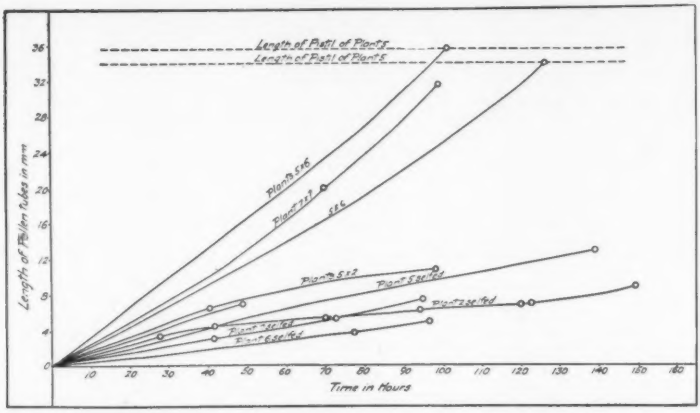

FIGURE 3.—Growth curves of pollen tubes from pistils of plants of *Nicotiana angustifolia*. Plants 5 and 2 are cross-sterile with each other.

growth becomes faster and faster as they approach the ovary. Since no points were noted on the crossed pistils during the early part of the progress of the tubes, the curves as drawn are not exactly like those of figure 1, but this difference has no real basis.

One of the curves of figure 3 is from an incompatible cross. It is practically the same as the self upon the same plant.

Many other curves have been made, but these are fair samples. All show the same type of growth. Selfs made during the active part of the flowering season always produce straight-line growth curves. The growth of the tubes is at a steady rate throughout the length of the style traversed. The same is true of incompatible crosses. Toward the wane of the flowering season, however, the type of curve always changes until it resembles that of a compatible cross, though there remains a slight difference. The growth is more rapid than that from mid-season selfings, but the velocity in these cases is almost constant while in compatible crosses there is an acceleration.

SUMMARY AND DISCUSSION

The following general conclusions may be drawn from the data reported in this paper.

1. Pollen grains of the four species of Nicotiana used in these investigations germinate on many artificial media as well as on the stigmas of the plants themselves, but the length of pollen tube obtained is never over .6 mm and usually is from .1 mm to .2 mm.

2. Though pollen tubes are emitted freely on artificial media, their growth is unlike that obtaining under natural conditions. There the growth either remains constant or becomes more rapid, but on artificial media the latter part of the growth is markedly retarded.

3. Pollen-tube chemotropism was not observed, but since the presence of gynæcium parts in the medium caused a longer tube to be produced, this negative result may have been due to rapid diffusion of the chemical stimulants.

4. It is possible to obtain pollen-tube penetration followed by fertilization in a decapitated style by the use of a proper germinating medium.

5. Pollen grains germinate as well on the stigmas of flowers of the same plant as they do on the stigmas of flowers of other plants with which they are compatible.

6. From 5 to 10 times as many pollen tubes are produced as are necessary to fecundate the ovules.

7. The pollen tubes produced after a selfing are indistinguishable in size from the pollen tubes produced after a cross, when pollen tubes of the same length are measured.

8. Though there is variation in the length of the pollen tubes both after a self-pollination and after a cross-pollination, this variation seems to be due wholly to differences in the rate of germination of the pollen grains or to other causes which are environmental in nature and not to any differences in gametic constitution between the pollen grains themselves.

9. Pollen tubes produced after self-pollinations grow steadily and apparently normally, but do not reach the ovary before the flower decays because this growth is slow. Length plotted against time is a straight line.

10. Pollen tubes produced after a compatible cross start their growth at about the same rate as the pollen tubes produced after selfing; but the growth becomes constantly more and more rapid. Length plotted against time produces a curve that resembles that of an autocatalytic reaction.

11. Cross-sterile combinations resemble selfings in the rate of pollen-tube growth.

12. At the wane of the flowering season rate of growth of self pollen tubes becomes more rapid, though there is little evidence of acceleration during their passage down the style.

These results appear to us to show that the pollen tubes in a selfed

pistil are not inhibited in their growth by substances secreted in that pistil, but rather that a substance or substances are secreted in the pistil after a compatible cross which accelerate growth, and that the direct cause of this secretion is a catalyser which the pollen-tube nucleus is able to produce because the zygotic constitution of the plant producing it is different in certain particular hereditary factors from that of the plant on which it is placed. Since pollen-tube growth is intercellular, it may be that some cytolysis occurs, but in the main there appears to be some local reaction between the pollen-tube nucleus and the contiguous cells of the style which produces or makes serviceable the nutrients necessary for tube growth. The action must be local because the presence of compatible pollen tubes does not accelerate the growth of self pollen tubes. The reaction must be mutual because one cannot account for the peculiar behavior of self-sterile plants in crosses if one interprets pollen-tube growth as a simple parasitism.

The action in the case of self pollen tubes or where incompatible crosses are made, is accounted for by the likeness of the parents in the effective hereditary factors postulated. Some action must take place in these cases because growth occurs but this is the action on which the other phenomenon is built. It does not seem to be in the nature of an inhibition because the growth of self pollen tubes is constant from the beginning to the end of the growth. If any change in rate of growth occurs it is a slight acceleration. Nor does this growth appear at all analogous to immunity phenomena as has been suggested by COMPTON (1913) and STOUT (1916). At least if there is similarity, the current theories of immunity do not serve to make the matter any clearer.

The change in rate of tube growth in selfed pistils toward the end of the flowering season we hold to be a phenomenon apart from those just discussed. Here instead of a mutual reaction between active cells, there seems to be more of a parasitism. The pollen tube is active, but the style cells are inactive. The active pollen tube, then, feeds on the broken down cells of the style. Our evidence of this is not conclusive, but it is very suggestive that the pollen-tube growth in this pseudo-fertility, as we have called it, seems to be merely faster throughout the whole period of its growth rather than increasingly rapid. It would seem that if this were the case, then where there is pseudo cross-fertility between a plant in active flower and a plant at the wane of the flowering period, reciprocal crosses would not give the same result as was maintained in our earlier paper. But our contention there was that plants in the height of their flowering period give like results in reciprocal crosses.

Moreover in cases where pseudo cross-fertility was noted, with perhaps a very few doubtful ones, the plants used in reciprocal crosses were at about the same flowering stage. We have since made a few incompatible crosses (10), however, between plants at the most active part of their flowering season and plants at the extreme end of their flowering season. There was, and we may admit we expected it, a difference between the reciprocals. Some fertility was shown when the old plants were used as pistillate parents, there was none in the reciprocals.

LITERATURE CITED

ADAMS, J., 1916 On the germination of the pollen grains of apple and other fruit trees. Bot. Gaz. **61**: 131-147.

AMICI, G. B., 1830 Note sur la mode d'action du pollen sur la stigmate. Ann. sci. nat. **21**: 329-332.

ANDRONESCU, D. I., 1915 The physiology of the pollen of *Zea mays* with special reference to vitality. Thesis: Univ. of Illinois. pp. 1-36. Champaign: Pub. by author.

CORRENS, C., 1889 Culturversuche mit dem Pollen von *Primula acaulis* Lam. Ber. d. d. bot. Gesell. **7**: 265-272.

1912 Selbststerilität und Individualstoffe. Festschr. d. math.-nat. Gesell. zur 84. Versamml. deutsch. Naturforscher u. Ärzte Münster i. W. pp. 1-32.

COUPIN, HENRI, 1906 Sur l'action de quelques alcaloïdes à l'égard des tubes polliniques. Compt. rend. Acad. Sci. Paris **142**: 841-843.

DEGAGNY, CHARLES, 1886 Sur le tube pollinique, son rôle physiologique. Compt. rend. Acad. Sci. Paris **102**: 230-231.

EAST, E. M., and PARK, J. B., 1917 Studies on self-sterility I. The behavior of self-sterile plants. Genetics **2**: 505-609.

GREEN, J. R., 1894 Researches on the germination of the pollen grain and the nutrition of the pollen tube. Phil. Trans. Roy. Soc. London B **185**: 385-409.

HANSGIRG, A., 1897 Beiträge zur Biologie und Morphologie des Pollens. Sitzber. kgl. Ges. d. Wiss. Prague **23**: 1-76.

JOST, L., 1905 Zur Physiologie des Pollens. Ber. d.d. bot. Gesell. **23**: 504-515.

1907 Über die Selbststerilität einiger Blüten. Bot. Ztg. **65**: 77-117.

LIDFORSS, BENGT, 1896 Zur Biologie des Pollens. Jahrb. f. wiss. Bot. **29**: 1-38.

1899 Über den Chemotropismus der Pollenschläuche. Ber. d. d. bot. Gesell. **17**: 236-242.

MANGIN, LOUIS, 1890 Sur la callose, nouvelle substance fondamentale existant dans la membrane. Compt. rend. Acad. Sci. Paris **110**: 644-647.

MARTIN, J. N., 1913 The physiology of the pollen of *Trifolium pratense*. Bot. Gaz. **56**: 112-156.

MIYOSHI, MANABU, 1894 Über Reizbewegungen der Pollenschläuche. Flora **78**: 76-93.

MOLISCH, HANS, 1893 Zur Physiologie des Pollens. Sitzungsber. Weiner Akad. Wiss., math.-naturw. Kl. **102**: 423-448.

RITTINGHAUS, P., 1887 Einige Beobachtungen über das Eindringen der Pollenschläuche ins Leitgewebe. Verh. nat. Vereines Rheinland **43**: 105-122.

STOUT, A. B., 1916 Self- and cross-pollinations in *Cichorium intybus* with reference to sterility. Mem. N. Y. Bot. Gard. **6**: 333-454.

STRASBURGER, E., 1886 Über fremdartige Bestäubung. Jahrb. f. wiss. Bot. **17**: 50-98.
VAN TIEGHEM, PH., 1869 Recherches sur la végétation libre du pollen et de l'ovule. Ann. sc. nat. V, **12**: 312.
TOKUGAWA, Y., 1914 Zur Physologie des Pollens. Jour. Coll. Sci. Tokyo **35**: 1-35.
TOMASCHEK, ANTON, 1882 Das Bewegungsvermögen der Pollenschläuche und Pollenpflanzen. Sitzber. Wien. Akad. Wiss. **84** (1881): 612-615.

ON THE NATURE OF SIZE FACTORS

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In a recent paper on the inheritance of stature in man, DAVENPORT (1917) discusses the relative importance of factors which affect the growth of the body as a whole and factors which affect only particular parts. He finds the latter more important in his data. He quotes results of CASTLE and of PEARSON in which the reverse was the case. The present paper is a further study of Prof. CASTLE's results, designed to illustrate a method of analysis as well as to bring out certain conclusions.

CASTLE (1914) gives all the correlations between five bone measurements in a stock of rabbits. He used length and breadth of skull, and lengths of humerus, femur and tibia. The measurements were those taken by MACDOWELL (1914) in his study of the inheritance of size in rabbits and are described by him in detail. This stock of rabbits was, unfortunately for the present purpose, of a rather heterogeneous character. It includes the offspring of a cross between a large and a small race and the back-cross of these with the small race. The inclusion of these two groups would tend to exaggerate the apparent importance of general size factors.

Both sexes are also combined. In three of the measurements this makes no appreciable difference. The average differences for length and width of skull and length of humerus were .001 cm, .031 cm and .007 cm, respectively, in favor of the females. The males, however, had distinctly longer hind legs. The average excess was .392 cm for the femur and .321 cm for the tibia. Thus the influence of sex must be taken into account where femur and tibia are involved.

In the following table, CASTLE's ten original correlations are given in the first column. The probable errors are small owing to the large size of the correlations as well as to the number of observations (between 370 and 380 in all cases). The five following columns give all the correlations between measurements for samples in which a third measurement is constant. These were calculated by PEARSON's familiar

formula for correlation between a and b for constant c, ${}_cR_{ab} = \frac{r_{ab} - r_{ac}r_{bc}}{\sqrt{(1-r^2_{ac})\times(1-r^2_{bc})}}$. The next ten columns give all the correlations for samples in which two measurements are constant, while the last column gives the correlations with three measurements constant. In the table OM is length of skull (occipital to maxilla), Zp is width of skull (posteriorly across the zygomatic arch), and H, F and T are the lengths of humerus, femur and tibia.

	r	OM^R	Zp^R	H^R	F^R	T^R	OM–Zp^R	OM–H^R	OM–F^R	OM–T^R
OM–Zp	.750 ± .015	. . .	. . .	.502	.496	.538	. . .	.	. .	. . .
H	.743 ± .016	. . .	.485	. . .	.275	.431	. . .	. . .	.	. . .
F	.760 ± .015	. . .	.519	.356	. .	.432	. . .	. . .	. . .	. . .
T	.701 ± .018		.416	.276	.147	. .	. . .			.
Zp–H	.675 ± .019	.266	. . .		.254	.334	. . .	.	.145	.135
F	.674 ± .019	.242	. . .	.250	. .	.282		.088		.067
T	.658 ± .020	.280		.275	.211	. .	. . .	.162	.160	
H –F	.857 ± .009	.671	.737	.	.	.567	.649	. . .	.	.467
T	.791 ± .013	.566	.628	. . .	.211	.	.531	. . .	.181	. . .
F –T	.858 ± .009	.700	.746	.571		. . .	.679	.524	. . .	. . .

	Zp–H^R	Zp–F^R	Zp–T^R	HF^R	HT^R	FT^R	R (three constant factors)
OM–Zp	. . .	. . .	. . .	.456	.502	.481	.448
H	. . .	.176	.316	. . .	. . .	.252	.172
F	.274	. . .	.345	. .	.251	. . .	.224
T	.163	.051		.095			.022
Zp–H	. . .	. . .	. . .	.		.218	.119
F	. . .	.	. . .	. .	.118	.	.004
T	. . .		. .	.166		.	.136
H –F	. .	.	.517	. .		. .	.463
T	. . .	.173	. . .	.	. .	.	.161
F –T	.537	. .	. .	. .	.	. . .	.517

The strikingly high correlations between skull and leg measurements were noted by CASTLE as evidence of the overwhelming importance of *general* factors for growth in this stock of rabbits.

Inspection of the primary correlations in the table shows that skull width (Zp) has very much more independent variability than the length of the skull (OM) and that the length of the tibia (T) has more independent variability than the length of humerus (H) or femur (F).

For further analysis, a study of the correlations with three measurements constant is very instructive. Such a population of rabbits should

be nearly free from variations due to general size factors. Calculation shows that with 75 percent of the variation (as measured by squared standard deviation) due to general size factors in the original population, only about 23 percent should be due to such factors within a sample in which three measurements are constant.

Inspection of the ten correlations with three factors constant (last column) shows that all are positive but only three exceed .22. Length and breadth of skull have a correlation of .45 even though all three leg bones are constant. Humerus and femur (homologous bones of different limbs) have a correlation of .46 when both skull measurements and *tibia* are constant and finally femur and tibia (bones of the same limb) have a correlation of .52, although both skull measures and *humerus* are constant. These three correlations suggest the existence of growth factors which affect the size of the skull independently of the body, others which affect similarly the length of homologous long bones apart from all else, and others which affect similarly bones of the same limb. In the last case we know directly of one factor, viz., sex, as has already been stated. It is to be noted especially that humerus and tibia (non-homologous and on different limbs) show very little correlation (.16) when the skull measures and *femur* are constant. Indeed the correlation is very small (.21) when the femur is the only constant measurement. The low correlation between a skull dimension and a leg bone when the other skull dimension and one or both of the other leg bones is constant, is not surprising. It is hardly worth considering the differences in magnitude of these small correlations ranging from .00 to .22 but it may be noted that there are slight indications that brachicephaly is associated with long tibia but short femur.

The correlations with two constant measures bear out the previous results in all respects. The only other point which need be noted is that humerus and tibia show considerable correlation (.53) when both skull measures are constant. This is in harmony with correlation found between the head measures with all three leg bones constant, indicating the existence of separate factors for growth of skull and growth of the rest of the body. The correlations with one constant factor fall in line with the interpretation already cited but give less clear-cut results owing to the larger part played by general growth factors. It is worth noting that the femur seems to be the most closely related to general growth of any of the measurements, while the width of the skull is the least. All of the original correlations are over .65. On making the femur constant all but one are reduced to below .28 while that one, the corre-

lation between length and breadth of skull, falls from .750 to .496. On the other hand when the skull breadth is constant, no correlation falls below .41 and the correlations between the long bones are all over .62.

It is of interest to attempt to assign definite values to the different classes of growth factors which are indicated. The following kinds may conveniently be distinguished, granting, of course, that no sharp lines can really be drawn.

Factors which affect:

a. General size (all parts of the skeleton alike).
b. Size of skull only, but all skull bones alike.
c. Length of leg bones only, but these alike.
d. Length of bones of hind limbs only, but these alike.
e. Length of homologous leg bones only, but these alike.
f. Each part independently.

A rough analysis can be made by use of the following proposition. Let X and Y be two characters whose variations are determined in part by certain causes A, B, C, etc., which act on both and in part by causes which apply to only one or the other, M and N respectively. These causes are assumed to be independent of each other. Represent by small letters, a, b, c, etc., the proportions of the variation of X determined by these causes and by a^1, b^1, c^1, etc., the proportions in the case of Y. The extent to which a cause determines the variation in an effect is measured by the proportion of the squared standard deviation of the latter for which it is responsible. This follows from the proposition that the squared standard deviations due to single causes acting alone may be combined by simple addition to find the squared standard deviation of an array in which all causes are acting, provided the causes are independent of each other, i.e., $\sigma^2_{A+B+C} = \sigma^2_A + \sigma^2_B + \sigma^2_C$.

Effects	Causes					
	A	B	C	D	M	N
X	a	b	c	d	m	
Y	a^1	b^1	c^1	d^1		n^1

As a, b, etc., are the proportions of the variation of X which are determined by the various causes

$$a + b + c + d \ldots\ldots + m = 1$$
$$a^1 + b^1 + c^1 + d^1 \ldots\ldots + n^1 = 1$$

It is easy to demonstrate the following proposition in regard to the correlation between X and Y.

$$r_{xy} = \pm \sqrt{aa^1} \pm \sqrt{bb^1} \pm \sqrt{cc^1} \ldots$$

Where a given cause as A produces effects in the same direction in X and Y the sign of the term $\sqrt{aa^1}$ is +. Where the effects are in opposite directions the sign is —.

Applying this method to the rabbit data, the following factors may be distinguished.

Growth factors

Variations in	General	Skull	Legs	Hind legs	Proximal leg bones	Special
OM	a	o	....	.....	.....	m
Zp	a^{I}	b^{I}	....	.....	.....	n^{I}
H	a^{II}	..	c^{II}	.....	e^{II}	o^{II}
F	a^{III}	..	c^{III}	d^{III}	e^{III}	p^{III}
T	a^{IV}	..	c^{IV}	d^{IV}	.....	q^{IV}

The ratios of a^{I} to a and of a^{IV} and a^{III} to a^{II} are easily calculated.

(1) $r^2_{OM\text{-}H} = aa^{\text{II}} = .5520$
(2) $r^2_{Zp\text{-}H} = a^{\text{I}}a^{\text{II}} = .4556$
(3) $r^2_{OM\text{-}F} = aa^{\text{III}} = .5776$
(4) $r^2_{Zp\text{-}F} = a^{\text{I}}a^{\text{III}} = .4543$
(5) $r^2_{OM\text{-}T} = aa^{\text{IV}} = .4914$
(6) $r^2_{Zp\text{-}T} = a^{\text{I}}a^{\text{IV}} = .4330$

The three values of $\frac{a^{\text{I}}}{a}$ derived by dividing (2) by (1), (4) by (3) and (6) by (5) are .8254, .7865 and .8812, with an average of .8310. Thus general growth is only 83 percent as important in determining variation of the width of the skull as it is in determining variations in skull length. By dividing (3) by (1) and (4) by (2) we get two values of $\frac{a^{\text{III}}}{a^{\text{II}}}$, 1.0464 and .9971, with an average of 1.0218. By dividing (5) by (1) and (6) by (2) we get two values of $\frac{a^{\text{IV}}}{a^{\text{II}}}$, .8902 and .9504, with an average of .9203. Thus as regards the proportion of their variation determined by general growth factors,

$$Zp : OM = .8310 : 1.0000$$
$$T : F : H = .9203 : 1.0218 : 1.0000$$

The skull measurements cannot be compared with the leg measurements without further assumptions. The following results are of use in this connection:

$$_{H}R_{OM\text{-}Z\flat} = \frac{r_{OM\text{-}Z\flat} - \sqrt{aa^{III}}\sqrt{a^{I}a^{II}}}{\sqrt{(1-r^{2}_{OM\text{-}H})(1-r^{2}_{Z\flat\text{-}H})}} = .502 \therefore \sqrt{aa^{I}} = \frac{.5021}{a^{II}},$$

$$_{F}R_{OM\text{-}Z\flat} = \frac{r_{OM\text{-}Z\flat} - \sqrt{aa^{III}}\sqrt{a^{I}a^{III}}}{\sqrt{(1-r^{2}_{OM\text{-}F})(1-r^{2}_{Z\flat\text{-}F})}} = .496 \therefore \sqrt{aa^{I}} = \frac{.5010}{a^{II}},$$

$$_{T}R_{OM\text{-}Z\flat} = \frac{r_{OM\text{-}Z\flat} - \sqrt{aa^{IV}}\sqrt{a^{I}a^{IV}}}{\sqrt{(1-r^{2}_{OM\text{-}T})(1-r^{2}_{Z\flat\text{-}T})}} = .538 \therefore \sqrt{aa^{I}} = \frac{.5010}{a^{II}}.$$

$\sqrt{aa^{I}} = \frac{.5014}{a^{II}}$ as the average of three determinations. Similarly from $_{OM}R_{HT}$ and $_{Z\flat}R_{HT}$ we get $\sqrt{a^{II}a^{IV}} = \frac{.5208}{a}$ or $\frac{.5320}{a}$, with $\sqrt{a^{II}a^{IV}} = \frac{.5264}{a}$ as the average of two determinations. Now $\sqrt{aa^{I}}$ cannot exceed $r_{OM\text{-}Z\flat}$ (which equals $\sqrt{aa^{I}} + \sqrt{bb^{I}}$). The maximum value, $\sqrt{aa^{I}} = .750$, is on the assumption that $\sqrt{bb^{I}} = 0$. Similarly $\sqrt{a^{II}a^{IV}}$ can have the maximum value .791 but only on the assumption that $\sqrt{c^{II}c^{IV}} = 0$.

As $a^{I} = .831\ a$, $\sqrt{aa^{I}} = a\sqrt{.831}$,

and the maximum value of a is therefore $\frac{.750}{\sqrt{.831}} = .823$.

Similarly the maximum value of a^{II} is $\frac{.791}{\sqrt{.920}} = .825$

From the equation $\sqrt{aa^{I}} = \frac{.5014}{a^{II}}$, the minimum value of $\sqrt{aa^{I}}$ is evidently $\frac{.5014}{.825} = .609$ (assuming $\sqrt{c^{II}c^{IV}} = 0$), and similarly the minimum value of $\sqrt{a^{II}a^{IV}}$ is $\frac{.5264}{.825} = .638$ (assuming $\sqrt{bb^{I}} = 0$).

These maximum and minimum possible values can be tabulated thus:

	No general skull factors ($\sqrt{bb^{I}} = 0$)	No general leg factors ($\sqrt{c^{II}c^{IV}} = 0$)
$\sqrt{aa^{I}}$	.750	.609
$\sqrt{a^{II}a^{IV}}$	.638	.791

The partial correlation between length and width of skull for constant humerus, femur and tibia ($_{H\text{-}F\text{-}T}R_{OM\text{-}Z\flat} = .448$), shows clearly that there are factors for skull size affecting length and breadth alike, while the partial correlation between humerus and tibia for constant

length and width of skull ($_{OM\text{-}Zp}R_{H\text{-}T} = .531$), shows that there are factors affecting the leg bones in common. Thus neither of the extreme assumptions, $\sqrt{bb^{I}} = 0$ and $\sqrt{c^{II}\,c^{IV}} = 0$, is tenable. The assumption that $\sqrt{aa^{I}}$, has a value about half way between the extremes .750 and .609,—viz., .679, should give satisfactory results. On this basis $a = .746$ and a^{I}, a^{II}, a^{III} and a^{IV} are easily calculated.

$a = .746$ $\qquad \sqrt{aa^{I}} = .679$

$a^{I} = .746 \times .831 = .620$ $\qquad \sqrt{a^{II}a^{IV}} = .707$

$$a^{II} = \frac{.5014}{.679} = .738$$

$a^{III} = .738 \times 1.022 = .754$

$a^{IV} = .738 \times .920 = .679$

As $r_{OM\text{-}Zp} = \sqrt{aa^{I}} + \sqrt{bb^{I}} = .750$,

$\sqrt{bb^{I}} = .750 - .679 = .071$ (maximum = .141, minimum = 0).

As $r_{HT} = \sqrt{a^{II}\,a^{IV}} + \sqrt{c^{II}\,c^{IV}} = .791$,

$\sqrt{c^{II}\,c^{IV}} = .791 - .707 = .084$ (minimum = 0, maximum = .153).

As $r_{HF} = \sqrt{a^{II}\,a^{III}} + \sqrt{c^{II}\,c^{III}} + \sqrt{e^{II}\,e^{III}} = .857$,

$\sqrt{c^{II}\,c^{III}}$ cannot differ much from $\sqrt{c^{II}\,c^{IV}}$, and .080 should be a fair estimate.

Thus $\sqrt{e^{II}\,e^{III}} = .857 - .747 - .080 = .030$

Similarly $r_{FT} = \sqrt{a^{III}\,a^{IV}} + \sqrt{c^{III}\,c^{IV}} + \sqrt{d^{III}\,d^{IV}} = .858$. Assigning the value .080 to $\sqrt{c^{III}\,c^{IV}}$ we get $\sqrt{d^{III}\,d^{IV}} = .858 - .717 - .080 = .061$.

The following table is derived from these results.

Relative importance of different classes of size factors of five bone lengths.

	General size	Skull	Legs	Hind legs	Proximal leg bones	Special	Total
OM	.746	.077	....	....	....	.177	1.0000
Zp	.620	.065	....	....	....	.315	1.0000
H	.739	....	.083	....	.030	.148	1.0000
F	.755	....	.085	.064	.030	.066	1.0000
T	.680	....	.076	.058	....	.186	1.0000

SUMMARY

Analysis of the relations between five bone lengths in a population of rabbits shows that most differences between individuals are those which

involve the size of the body as a whole. There is, however, a certain amount of variation of each bone length independently of all others measured and there are also groups of bones which vary together independently of the rest of the body. Skull length and breadth on one hand, and the three leg bones on the other, are two such groups. Again the bones of the hind leg, femur and tibia, form a group subject to common influences which do not affect the humerus, a bone of the foreleg, and finally femur and humerus, homologous bones in hind and foreleg, vary together independently of the tibia. A mode of estimating the relative importance of these different kinds of growth factors is presented and applied to the rabbit data.

LITERATURE CITED

CASTLE, W. E., 1914 The nature of size factors as indicated by a study of correlation. Carnegie Inst. Wash. Publ. No. 196, pp. 51-55.

DAVENPORT, C. B., 1917 Inheritance of stature. Genetics **2**: 313-389.

MAC DOWELL, E. C., 1914 Size inheritance in rabbits. Carnegie Inst. Wash. Publ. No. 196, pp. 7-49.

SOME APPLICATIONS OF MATHEMATICS TO BREEDING PROBLEMS III

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TABLE OF CONTENTS

PAGE

INTRODUCTION 375
PART I. RANDOM MATING 375
a. Linkage r in each set of gametes 375
b. Linkage r in one set of gametes and r' in the other 378
PART II. SELECTION OF DOMINANTS WITH RESPECT TO ONE OF THE PAIRS OF CHARACTERS—LINKAGE r IN SETS OF GAMETES 381
PART III. SELF-FERTILIZATION 386
a. Linkage r in each set of gametes 386
b. Linkage r in one set of gametes and r' in the other set 388
LITERATURE CITED 389

INTRODUCTION

Professor H. S. JENNINGS (1916, 1917) has published two papers in GENETICS, giving numerical results for different systems of breeding in which the inheritance of Mendelian factors is in question. The first paper deals with one-factor problems, the second with two-factor problems. The present author has dealt with more general one-factor problems (ROBBINS 1917, 1918) suggested by JENNINGS's work. Similarly the present paper follows JENNINGS's lead in two-factor problems.

Part I gives the results for random mating for the most general problem of two linked factors. Part II is a less satisfactory solution of the problem of selection with regard to one of two linked factors. Part III gives the results for the general problem of self-fertilization.

The work of JENNINGS on random mating shows how useful it is to deal with the four kinds of gametes involved instead of the ten kinds of individuals. Of course, at any stage of the game we can find the proportions of the different types of individuals from our knowledge of the gametes of the parents.

I. RANDOM MATING

a. Linkage r in each set of gametes

Let A, a represent respectively the dominant and recessive factors of

a simple Mendelian pair; similarly let B, b represent respectively the dominant and recessive factors of a second simple Mendelian pair. With respect to these two sets of factors we will have four types of gametes. Let p_n, q_n, s_n, t_n be respectively proportional to the number of AB, Ab, aB, ab gametes that will combine to produce the $(n+1)$th generation. A zygote will be represented by the juxtaposition of the letters representing the gametes which unite to produce the zygote. If a zygote produces r gametes of each of the types which united to produce it, for each gamete of the type obtained by interchanging a pair of the factors in the original gametes, there is said to be a linkage r between the factors. For instance, if a zygote $ABab$ produces gametes in the proportion $rAB + Ab + aB + rab$, the factors have a linkage r. Using this notation, JENNINGS has expressed the proportions of gametes in the $(n+1)$th generation in terms of those in the nth generation in his table 9 (JENNINGS 1917, p. 144):

$$1)\quad \begin{cases} p_{n+1} = (r+1)\, p_n\, (p_n + q_n + s_n) + r\, p_n\, t_n + q_n\, s_n, \\ q_{n+1} = (r+1)\, q_n\, (p_n + q_n + t_n) + r\, q_n\, s_n + p_n\, t_n, \\ s_{n+1} = (r+1)\, s_n\, (p_n + s_n + t_n) + r\, q_n\, s_n + p_n\, t_n, \\ t_{n+1} = (r+1)\, t_n\, (q_n + s_n + t_n) + r\, p_n\, t_n + q_n\, s_n. \end{cases}$$

The form of these equations is decidedly simplified if we choose $p_n \ldots . t_n$ such that

$$2)\quad p_n + q_n + s_n + t_n = 1,$$

and use the notation $\Delta_n = q_n s_n - p_n t_n$. Then if we replace n by $n-1$ in equations 1) we obtain the following set:

$$3)\quad \begin{cases} p_n = p_{n-1} + \Delta_{n-1}/(1+r), \\ q_n = q_{n-1} - \Delta_{n-1}/(1+r), \\ s_n = s_{n-1} - \Delta_{n-1}/(1+r), \\ t_n = t_{n-1} + \Delta_{n-1}/(1+r). \end{cases}$$

These equations can be solved with little difficulty. From the first of equations 3) we have

$$4)\quad p_n = \frac{\Delta_{n-1}}{1+r} + \frac{\Delta_{n-2}}{1+r} + \ldots\ldots + \frac{\Delta_0}{1+r} + p_0.$$

If we can find Δ_n in terms of n we will have the desired solution for p_n. Calculating Δ_n from equations 3), i.e., forming $q_n s_n - p_n t_n$ and using Δ_{n-1} for $q_{n-1} s_{n-1} - p_{n-1} t_{n-1}$, we have

$$\Delta_n = \Delta_{n-1} - \frac{\Delta_{n-1}}{1+r} = \frac{r}{1+r} \cdot \Delta_{n-1}.$$

Whence,

5) $$\Delta_n = \left(\frac{r}{1+r}\right)^n \cdot \Delta_0 .$$

Substituting from 5) into 4) gives,

$$p_n = \frac{\Delta_0}{1+r}\left[\left(\frac{r}{1+r}\right)^{n-1} + \left(\frac{r}{1+r}\right)^{n-2} + \ldots\ldots + \frac{r}{1+r} + 1\right] + p_0 .$$

The bracket is a geometric progression. Summing it, p_n takes the closed form

$$p_n = \frac{\Delta_0}{(1+r)^n}[(1+r)^n - r^n] + p_0 .$$

Similar calculation for q_n, s_n, t_n gives us finally the set of solutions,

6) $$p_n = p_0 + \Delta_0\left[1 - \left(\frac{r}{1+r}\right)^n\right],$$

7) $$q_n = q_0 - \Delta_0\left[1 - \left(\frac{r}{1+r}\right)^n\right],$$

8) $$s_n = s_0 - \Delta_0\left[1 - \left(\frac{r}{1+r}\right)^n\right],$$

9) $$t_n = t_0 + \Delta_0\left[1 - \left(\frac{r}{1+r}\right)^n\right],$$

in which $\Delta_0 = q_0 s_0 - p_0 t_0$.

To get the zygotic composition of the $(n+1)$th generation it is only necessary to substitute these values for $p_n \ldots . t_n$ in JENNINGS's (1917) table (6).

DISCUSSION. 1. The sum $p_n + q_n$ represents the gametes *AB* and *Ab* in the nth generation; i.e., all the gametes having the factor *A*. Similarly $s_n + t_n$ represents all the gametes with the factor *a*. It is well known that for a single factor the proportions of dominants, recessives and heterozygous individuals is fixed after the first random mating. This is due to the fact that the proportions of dominant and recessive gametes never changes in random mating. Then in our problem we should expect $p_n + q_n$ to be constant, and our equations 6) and 7) show that $p_n + q_n = p_0 + q_0$. Similarly we have three other check equations.

2. *From equations* 6) *to* 9) *it is evident that the value of* Δ_0 *is quite important. Any two sets of initial conditions give results differing from the initial conditions by the same amounts if and only if* Δ_0 *is the same for both.*

3. *In the case* $\Delta_0 = 0$, *the proportions are fixed from the beginning.* This is shown by equations 6) to 9). *The only other case in which the proportions are fixed is that of complete linkage,* $r = \infty$. Then we have

$r/(1+r)$ approaches unity as a limit, and equation 6) becomes, $p_n = p_0$, and similarly, $q_n = q_0$, $s_n = s_0$, $t_n = t_0$. *It is also evident that the case of complete linkage is in essence a one-factor problem and that therefore the proportions should be fixed.*

4. *The results for independent factors are obtained by setting* $r = 1$:

$$p_n = p_0 + \Delta_0 (1 - \tfrac{1}{2}^n),$$
$$q_n = q_0 - \Delta_0 (1 - \tfrac{1}{2}^n),$$
$$s_n = s_0 - \Delta_0 (1 - \tfrac{1}{2}^n),$$
$$t_n = t_0 + \Delta_0 (1 - \tfrac{1}{2}^n).$$

b. Linkage r in one set of gametes and r′ in the other

We have a much more general problem than the one above if we assume that the degree of linkage is different in the different sexes. Consider the problem with linkages r and r' any two positive integers. Let p_n, q_n, s_n, t_n be the gametic proportions in the set of gametes of linkage r and p_n', q_n', s_n', t_n' the same for the set of linkage r'. Then a study of the crosses involved gives the following recurrences:

10)
$$\begin{cases} p_n = \dfrac{p_{n-1} + p_n'_{-1}}{2} + \dfrac{d_{n-1}}{2(r+1)}, \\ q_n = \dfrac{q_{n-1} + q_n'_{-1}}{2} - \dfrac{d_{n-1}}{2(r+1)}, \\ s_n = \dfrac{s_{n-1} + s_n'_{-1}}{2} - \dfrac{d_{n-1}}{2(r+1)}, \\ t_n = \dfrac{t_{n-1} + t_n'_{-1}}{2} + \dfrac{d_{n-1}}{2(r+1)}, \end{cases}$$

11)
$$\begin{cases} p_n' = \dfrac{p_{n-1} + p_n'_{-1}}{2} + \dfrac{d_{n-1}}{2(r'+1)}, \\ q_n' = \dfrac{q_{n-1} + q_n'_{-1}}{2} - \dfrac{d_{n-1}}{2(r'+1)}, \\ s_n' = \dfrac{s_{n-1} + s_n'_{-1}}{2} - \dfrac{d_{n-1}}{2(r'+1)}, \\ t_n' = \dfrac{t_{n-1} + t_n'_{-1}}{2} + \dfrac{d_{n-1}}{2(r'+1)}, \end{cases}$$

in which

$$d_n = q_n s_n' - p_n t_n' + q_n' s_n - p_n' t_n.$$

Substituting from equation 11) into equation 10) we have,

$$12)\begin{cases} p_n = p_n' + \dfrac{(r'-r)\,d_{n-1}}{2(r+1)(r'+1)}, \\ q_n = q_n' - \dfrac{(r'-r)\,d_{n-1}}{2(r+1)(r'+1)}, \\ s_n = s_n' - \dfrac{(r'-r)\,d_{n-1}}{2(r+1)(r'+1)}, \\ t_n = t_n' + \dfrac{(r'-r)\,d_{n-1}}{2(r+1)(r'+1)}. \end{cases}$$

Substituting these values of $p_n \ldots . t_n$ into the equations 11) gives us,

$$13)\begin{cases} p_n' = p_{n-1}' + \nabla_{n-1}, \\ q_n' = q_{n-1}' - \nabla_{n-1}, \\ s_n' = s_{n-1}' - \nabla_{n-1}, \\ t_n' = t_{n-1}' + \nabla_{n-1}. \end{cases}$$

where $n>1$ and

$$\nabla_{n-1} = \frac{1}{2(r'+1)}\left[\frac{r'-r}{2(r+1)}d_{n-2} + d_{n-1}\right].$$

It is evident that we can solve our problem if we can find ∇_{n-1}, and this depends upon finding d_n. If we let $D_n = q_n'\ s_n' - p_n'\ t_n'$, detailed computation from equations 12) and 13) show that

$$d_n = 2\ D_n - \frac{(r'-r)d_{n-1}}{2(r+1)(r'+1)}, \text{ and } D_n = D_{n-1} - \nabla_{n-1}.$$

From these last equations we find that

$$d_n = d_{n-1}\left[\frac{r}{2(r+1)} + \frac{r'}{2(r'+1)}\right] \text{ for } n>1\,.$$

Whence

$$d_n = d_1.\,K^{n-1} \text{ where}$$

$$K = \frac{r}{2(r+1)} + \frac{r'}{2(r'+1)}.$$

Having d_n, we can calculate ∇_{n-1}, then solve equations 13) and finally equations 12).

$$p_n' - p_{n-1}' = \nabla_{n-1} = \frac{d_1\,r'K^{n-2}(r'+r+2)}{2(r'+1)(2rr'+r+r')}.$$

The solution of this equation is

14 a) $p_n' = c_1 - d_1\, r'\, K^{n-2}/2(r'+1), \qquad n>1.$

in which[1]

$$d_1 = q_1\, s_1' - p_1 t_1' + q_1'\, s_1 - p_1'\, t_1 \text{ and } c_1 = (p_1 + p_1' + d_1)/2.$$

Similarly,

14 b) $q_n' = c_2 + d_1\, r'\, K^{n-2}/2(r'+1),$

14 c) $s_n' = c_3 + d_1\, r'\, K^{n-2}/2(r'+1),$

14 d) $t_n' = c_4 - d_1\, r'\, K^{n-2}/2(r'+1),$

in which $c_2 = (q_1 + q_1' - d_1)/2$; $c_3 = (s_1 + s_1' - d_1)/2$; $c_4 = (t_1 + t_1' + d_1)/2$.

Substituting into equations 12) we have,

15)
$$\begin{cases} p_n = c_1 - d_1\, r\, K^{n-2}/2(r+1), \\ q_n = c_2 + d_1\, r\, K^{n-2}/2(r+1), \\ s_n = c_3 + d_1\, r\, K^{n-2}/2(r+1), \\ t_n = c_4 - d_1\, r\, K^{n-2}/2(r+1). \end{cases}$$

Discussion. 1. It is evident that these results should reduce to those in the previous problem, linkage r in each sex, if we set $r'=r$. This can be easily verified and serves as a check on the calculations. *Equations* 10) *and* 11) *show that however different the original proportions may be in the two sexes, they are identical after the first cross, i.e.,* $p_1=p_1'$, *etc., if the linkage is the same in both sexes.*

2. *The results for the case of complete linkage in one set of gametes is given by making* r' *infinite in the above formulae. This gives:*

$$p_n' = c_1 - d_1\, K^{n-2}/2,$$
$$q_n' = c_2 + d_1\, K^{n-2}/2,$$
$$s_n' = c_3 + d_1\, K^{n-2}/2,$$
$$t_n' = c_4 - d_1\, K^{n-2}/2,$$

in which $K = (2r+1)/2(r+1)$ *and* $c_1 \ldots c_4$ *are unchanged. The form of the equations for* $p_n \ldots t_n$ *does not change.*

3. *Setting* $r' = 1$ *will give the case of no linkage in one set of gametes. The equations become,*

$$p_n' = c_1 - d_1\, K^{n-2}/4,$$
$$q_n' = c_2 + d_1\, K^{n-2}/4,$$
$$s_n' = c_3 + d_1\, K^{n-2}/4,$$
$$t_n' = c_4 - d_1\, K^{n-2}/4,$$

where $K = (3r + 1)/4(r + 1)$. *Here again the form of the equations for* $p_n \ldots\ldots t_n$ *remains unchanged.*

[1] Of course we can express c_1 and d_1 in terms of the original data, $p_0 \ldots t_0$, but so expressed they are cumbersome. The simpler is c_1 which is given by

$$c_1 = (2\, p_0 + 2\, p_0' + d_0 + q_0 s_0 - p_0 t_0 + q_0' s_0' - p_0' t_0')/4.$$

4. *Equations* 14) *and* 15) *show that if* $d_1 = 0$, *the proportions will be fixed after one random mating. In this case the proportions will be independent of the degree of linkage.* This last statement can easily be verified by calculating $c_1 \ldots c_4$ in terms of $p_0 \ldots . t_0'$. The parts involving r and r' disappear—(see note 1).

5. *The limiting population:*

a. *The gametic proportions approach limiting values as n increases.*

b. *The limiting values are equal in the two sexes;* i.e., *the limits of* p_n, q_n, s_n, t_n *are respectively the limits of* p_n', q_n', s_n', t_n'. *In case r and r' are not both infinite the limits of* p_n, q_n, s_n, t_n *are respectively* c_1, c_2, c_3, c_4. In case of complete linkage the limits are respectively $c_1 - d_1/2$, $c_2 + d_1/2$, $c_3 + d_1/2$, $c_4 - d_1/2$.

c. *The limiting values are independent of the linkage factors r, r' except in the sense that complete linkage in both sexes gives limits different from those for any other case.* As was pointed out above, the case of complete linkage in both sexes is really a one-factor problem, and the proportions are fixed after one random mating.

d. *The limiting proportions must be such that if used as initial proportions the population would remain fixed;* this follows because if after the limiting proportions had been reached one more random mating changed the proportions, our notion of limiting proportions would be violated. We can therefore check our limits by forming d_1 using $p_1 = p_1' = c_1$, $q_1 = q_1' = c_2$, $s_1 = s_1' = c_3$, $t_1 = t_1' = c_4$. From point 4 in the discussion, d_1 should vanish, and detailed calculation will show that it does.

e. *In the limiting population, the proportion of A B gametes is the product of the proportions of A gametes and B gametes.* Symbolically this is expressed by the equation $c_1 = (c_1 + c_2)(c_1 + c_3)$, which may be easily verified.

Two striking facts stand out as a result of this discussion:

1. *In random mating, the effect of incomplete linkage between two factors is only temporary.*

2. *Continued random mating results in a population in which the distribution of B factors among the A and a factors is the same as the distribution of the b factors among the A and a factors.*

II. SELECTION OF DOMINANTS WITH RESPECT TO ONE OF THE PAIRS OF CHARACTERS—LINKAGE r IN BOTH SETS OF GAMETES

In this problem we select for breeding purposes only the zygotes which have the factor A. A study of the crosses involved gives the following recurrence relations:

$$16)\quad \begin{cases} p_n = [(r+1)\ p_{n-1} + \delta_{n-1}]/D_{n-1}, \\ q_n = [(r+1)\ q_{n-1} - \delta_{n-1}]/D_{n-1}, \\ s_n = [(r+1)\ s_{n-1}\ l_{n-1} - \delta_{n-1}]/D_{n-1}, \\ t_n = [(r+1)\ t_{n-1}\ l_{n-1} + \delta_{n-1}]/D_{n-1}, \end{cases}$$

In which $\delta_n = q_n\ s_n - p_n\ t_n$; $D_n = l_n\ (1 + L_n)(r+1)$; $L_n = s_n + t_n$ and $l_n = p_n + q_n$.

The method of solving these equations is analogous to the methods used in the earlier problems, but there is more detail and the results are less satisfactory. It is convenient to solve first for L_n and l_n. It is evident that L_n and l_n give the gametic composition of the nth generation for the one factor problem and we could compute them from this standpoint, but it is easy to calculate them from the equations 16). We have

$$s_n + t_n = (r+1)l_{n-1}(s_{n-1} + t_{n-1})/D_{n-1} = (s_{n-1} + t_{n-1})/(1 + L_{n-1}).$$

Since $s_n + t_n = L_n$,

$$L_n = L_{n-1}/(1 + L_{n-1}).$$

Then

$$17)\quad \frac{1}{L_n} = \frac{1 + L_{n-1}}{L_{n-1}} = 1 + \frac{1}{L_{n-1}} = 2 + \frac{1}{L_{n-2}} = \ldots = n + \frac{1}{L_0}.$$

Whence,

$$18)\quad L_n = L_0/(n\,L_0 + 1).$$

$$19)\quad l_n = 1 - L_n = \frac{(n-1)L_0+1}{n\,L_0+1} = \frac{L_n}{L_{n-1}}.$$

Combining equations 17) and 19) we get,

$$20)\quad \frac{1}{1 + L_n} = \frac{L_{n+1}}{L_n} = l_{n+1}.$$

Equation 20) enables us to write D_n in the simpler form,

$$21)\quad D_n = (r+1)l_n/l_{n+1}.$$

It is at once apparent that L_n approaches zero as n increases and hence s_n and t_n do likewise. Therefore l_n approaches unity as n increases.

The next step in the solution is to solve for δ_n. Computing δ_n from equations 16), i.e. calculating $q_n\ s_n - p_n\ t_n$ we get at once that

$$22)\quad \delta_n = \delta_{n-1}(r - L_{n-1})/D_{n-1}(1 + L_{n-1}).$$

Substituting from equations 20) and 21) for $1/(1+L_{n-1})$ and D_{n-1}, δ_n takes the form

$$\begin{aligned} \delta_n &= \delta_{n-1}\, l_n^2 (r - L_{n-1})/l_{n-1}(r+1), \\ &= \delta_{n-2}\ l_n^2\ l_{n-1}(r - L_{n-1})(r - L_{n-2})/(r+1)^2\ l_{n-2}, \\ &= \delta_0 . l_n . l_n . l_{n-1} \ldots\ldots\ l_1 (r - L_{n-1}) \ldots\ldots\ (r - L_0)/(r+1)^n . l_0 \end{aligned}$$

From equation 20) we have,

$$l_n\, l_{n-1} \ldots\ldots l_1 = L_n/L_0.$$

Then we can write,

$$23) \qquad \delta_n = \delta_0\ l_n L_n \prod_{i=0}^{i=n-1} (r-L_i)/\, l_0\, L_0\, (r+1)^n\,.$$

The first of equations 16) can be written

$$p_n - l_n\, p_{n-1}/l_{n-1} = \delta_{n-1}\, l_n/(r+1) l_{n-1}.$$

The solution of this equation satisfying the initial conditions is,

$$24) \qquad p_n = \frac{p_0\, l_n}{l_0} + \frac{l_n}{r+1}\left[\frac{\delta_{n-1}}{l_{n-1}} + \frac{\delta_{n-2}}{l_{n-2}} + \ldots\ldots + \frac{\delta_0}{l_0}\right].$$

Similarly the solutions of the other equations of set 16) are,

$$25) \qquad q_n = \frac{q_0\, l_n}{l_0} - \frac{l_n}{r+1}\left[\frac{\delta_{n-1}}{l_{n-1}} + \frac{\delta_{n-2}}{l_{n-2}} + \ldots\ldots + \frac{\delta_0}{l_0}\right].$$

$$26) \qquad s_n = \frac{s_0\, L_n}{L_0} - \frac{l_n}{r+1}\left[\frac{\delta_{n-1}}{l_{n-1}} + \frac{\delta_{n-2}}{l_{n-2}} + \ldots\ldots + \frac{\delta_0}{l_0}\right].$$

$$27) \qquad t_n = \frac{t_0\, L_n}{L_0} + \frac{l_n}{r+1}\left[\frac{\delta_{n-1}}{l_{n-1}} + \frac{\delta_{n-2}}{l_{n-2}} + \ldots\ldots + \frac{\delta_0}{l_0}\right].$$

This seems to be about the most compact form into which the solution can be put. It will probably be a matter of opinion whether these equations are worth writing down. Certainly if one desires the composition of each generation, repeated use of the recurrence relation is easiest. But if one wishes the tenth generation and does not care about the preceding ones, it seems that the solutions 24) to 27) may be more useful. It should be noted that L_n and l_n are very simple functions of n and can be calculated rapidly, and that successive values of δ_n come rather easily if we use equation 22).

DISCUSSION. 1. *As noted above, s_n and t_n approach zero as n increases.*

2. *The proportions can be fixed only in the trivial case where* $s_0 = t_0 = 0$. This is shown by equation 18).

3. *If $p_i/q_i = s_i/t_i$ for any value of i it is true for all values of i.* This follows from equation 22), since if $p_i/q_i = s_i/t_i$, then $\delta_i = 0$. *In this special case, the equations* 24) *to* 27) *reduce to*

$$p_n = p_0 l_n/l_0;\ q_n = q_0\, l_n/l_0;$$
$$s_n = s_0\, L_n/L_0;\ t_n = t_0\, L_n/L_0.$$

It is important to note that in this case, $\delta_i = 0$, the results are independent of the linkage factor. Furthermore we find that $p_n + s_n = p_0 + s_0$. This is readily shown as follows. From equations 24) and 26)

$$p_n + s_n = p_o\, l_n/l_o + s_o\, L_n/L_o \text{ when } \delta_o = 0;$$
$$= p_o l_n/l_o + s_o(1-l_n)/L_o$$
$$= l_n[p_o/l_o - s_o/L_o] + s_o/L_o.$$

Since $p_o/q_o = s_o/t_o$, then $p_o/(p_o + q_o) = s_o/(s_o + t_o)$; i.e., $p_o/l_o - s_o/L_o = 0$, and therefore

$$p_n + s_n = s_o/L_o.$$

Also since $p_o/(p_o + q_o) = s_o/(s_o + t_o)$, each fraction is equal to $(p_o + s_o)/(p_o + q_o + s_o + t_o) = \dfrac{p_o + s_o}{1}$. Therefore,

$$p_n + s_n = p_o + s_o.$$

This is an important fact. The sum $p_n + s_n$ represents the gametes with the factor B in the nth generation. We therefore have the conclusion, if $\delta_o = 0$, selection of dominants with respect to A does not interfere with random mating with respect to B, regardless of the degree of linkage between A and B.

4. *The case of complete linkage, $r = \infty$, gives the same equations for $p_n \ldots\ldots t_n$ as does $\delta_o = 0$. However, we do not have the other results that follow from $\delta_o = 0$.*

5. *The case of no linkage, $r = 1$, simplifies considerably because the continued product for δ_n (equation 23) can be summed when $r = 1$:*

$$\delta_n = \delta_o\, l_n\, L_n\, l_{n-1} \ldots . l_o/l_o\, L_n\, 2^n.$$
$$= \delta_o\, L_n^2/L_o^2 \cdot 2^n, \text{ (using equation 19)).}$$
$$\delta_n/l_n = \delta_o\, L_n^2/L_o^2 \cdot 2^n \cdot l_n.$$

Using equation 19) again, this becomes

$$\delta_n/l_n = \delta_o\, L_n\, L_{n-1}/L_o^2 \cdot 2^n.$$

From equation 17) we have

$$1/L_n - 1/L_{n-1} = 1.$$

Whence,

$$L_n \cdot L_{n-1} = L_{n-1} - L_n.$$

Substituting this value of $L_n\, L_{n-1}$ above, we have,

$$\delta_n/l_n = \delta_o(L_{n-1} - L_n)/L_o^2 \cdot 2^n.$$

Using this value of δ_n/l_n, equations 24) to 27) may be written,

28) $$p_n = \left(p_o + \frac{\delta_o}{2} + \frac{\delta_o\, l_o}{4\, L_o}\right)\frac{l_n}{l_o} - \left(S_{n-1} + \frac{L_{n-1}}{2^{n-1}}\right) \cdot \frac{l_n\, \delta_o}{4\, L_o^2}.$$

29) $$q_n = \left(q_o - \frac{\delta_o}{2} - \frac{\delta_o\, l_o}{4\, L_o}\right)\frac{l_n}{l_o} + \left(S_{n-1} + \frac{L_{n-1}}{2^{n-1}}\right) \cdot \frac{l_n\, \delta_o}{4\, L_o^2}.$$

30) $$s_n = \left(s_o - \frac{\delta_o}{2\, l_o} - \frac{\delta_o}{4}\right)\frac{L_n}{L_o} - \left(S_{n-1} - \frac{L_{n-1}}{2^{n-1}}\right) \cdot \frac{L_n\, \delta_o}{4\, L_o^2}.$$

31) $$t_n = \left(t_0 + \frac{\delta_0}{2\,l_0} + \frac{\delta_0}{4}\right)\frac{L_n}{L_0} + \left(S_{n-1} - \frac{L_{n-1}}{2^{n-1}}\right) \cdot \frac{L_n\,\delta_0}{4\,L_0^2} \cdot$$

in which $S_n = L_1/2 + L_2/4 + \ldots + L_n/2^n$.

The computation in this case is fairly simple and the formulae should be useful.

JENNINGS (1917) discusses this problem in section (26) of his paper. In the next to the last paragraph of this section he writes, "selection with reference to A and a is random mating with reference to B and b, if the two pairs are not linked." There is nothing in our equations 28) to 31) to suggest this, and as a matter of fact an example can easily be found for which this is not true. Suppose, for instance, that the breeding begins with a cross between $ABAb$ and $abab$ and suppose there is no linkage, $r = 1$. Then $p_0 = \frac{1}{4}$; $q_0 = \frac{1}{4}$; $s_0 = 0$; $t_0 = \frac{1}{2}$. From equations 16) or equations 28) to 31), or from JENNINGS's equations of table 16, we calculate,

$$p_1 = \tfrac{1}{4},\quad q_1 = 5/12,\quad s_1 = 1/12,\quad t_1 = \tfrac{1}{4},$$
$$p_2 = 17/64,\quad q_2 = 31/64,\quad s_2 = 5/64,\quad t_2 = 11/64.$$

In random mating with respect to B and b, the proportion of each type of gamete remains fixed. The proportion of B gametes is given by $p_n + s_n$. In the above example,

$$p_0 + s_0 = \tfrac{1}{4};\quad p_1 + s_1 = \tfrac{1}{3};\quad p_2 + s_2 = 11/32.$$

Thus we see that we do not have random mating with respect to B and b.

6. *The proportions approach limiting values as n increases.* As has already been mentioned, s_n and t_n approach zero. That p_n and q_n approach limiting values is apparent when we notice from equations 24) and 25) that each increases or decreases continuously and lies between zero and unity. The limits of p_n and q_n are

$$\lim_{n=\infty} p_n = \frac{p_0}{l_0} + \frac{1}{r+1}\left[\frac{\delta_0}{l_0} + \frac{\delta_1}{l_1} + \frac{\delta_2}{l_2} + \ldots\right],$$

$$\lim_{n=\infty} q_n = \frac{q_0}{l_0} - \frac{1}{r+1}\left[\frac{\delta_0}{l_0} + \frac{\delta_1}{l_1} + \frac{\delta_2}{l_2} + \ldots\right].$$

We can say very little about these values because of their complicated form. However, we may note this one fact: *the limits of p_n and q_n depend upon the value of r and $\delta_0 = 0$.* This is worth noting since it was not the case in random mating.

It may be worth while to state without proof that in case $r = 1$, p_n lies between the values $p_0/l_0 + \delta_0/2l_0$ and $p_0/l_0 + \delta_0/2l_0 + \delta_0/2L_0$. Also, the difference between these two expressions, $\delta_0/2L_0$, lies between zero and $\frac{1}{2}$.

Similar investigations can be carried through for the corresponding problem in which the linkage constant is different in the two sexes, but the results become complicated so rapidly that it would seem wiser to follow JENNINGS's method of repeated use of the recurrence relations.

III. SELF-FERTILIZATION

a. Linkage r in each set of gametes

In this problem we cannot deal with the types of gametes only. We must consider the different types of zygotes. We shall follow JENNINGS in letting c_n represent the proportion of the zygotes of the nth generation which have a composition indicated by $ABAB$, and use similar notation for other types as indicated in the following table:

$c_n = ABAB$		$i_n = ABAb$
$d_n = AbAb$	$g_n = ABab$	$j_n = ABaB$
$e_n = aBaB$	$h_n = AbaB$	$k_n = abAb$
$f_n = abab$		$l_n = abaB$

If we assume that

$$c_n + d_n + e_n + f_n + g_n + h_n + i_n + j_n + k_n + l_n = 1,$$

the recurrence relations for the problem are,

32) $c_n = c_{n-1} + r^2 g_{n-1}/R + h_{n-1}/R + (i_{n-1} + j_{n-1})/4,$

33) $d_n = d_{n-1} + g_{n-1}/R + r^2 h_{n-1}/R + (i_{n-1} + k_{n-1})/4,$

34) $e_n = e_{n-1} + g_{n-1}/R + r^2 h_{n-1}/R + (j_{n-1} + l_{n-1})/4,$

35) $f_n = f_{n-1} + r^2 g_{n-1}/R + h_{n-1}/R + (k_{n-1} + l_{n-1})/4,$

36) $g_n = 2[r^2 g_{n-1} + h_{n-1}]/R,$

37) $h_n = 2[g_{n-1} + r^2 h_{n-1}]/R,$

38) $i_n = 2r(g_{n-1} + h_{n-1})/R + i_{n-1}/2,$

39) $j_n = 2r(g_{n-1} + h_{n-1})/R + j_{n-1}/2,$

40) $k_n = 2r(g_{n-1} + h_{n-1})/R + k_{n-1}/2,$

41) $l_n = 2r(g_{n-1} + h_{n-1})/R + l_{n-1}/2,$

in which $R = 4(1 + r^2)$.

Adding 36) and 37) and using the notation $v = (r^2 + 1)/2(r + 1)^2$,

$$g_n + h_n = v(g_{n-1} + h_{n-1}).$$

Whence

42) $g_n + h_n = v^n(g_0 + h_0).$

Substituting from 42) into 38) gives,

$$i_n - i_{n-1}/2 = 2r(g_0 + h_0).v^{n-1}/R.$$

The solution of this equation is

43) $$i = \frac{g_0 + h_0 + 2i_0}{2^{n+1}} - \frac{g_0 + h_0}{2}.v^n.$$

Similarly,

44) $$j_n = \frac{g_0 + h_0 + 2j_0}{2^{n+1}} - \frac{g_0 + h_0}{2} \cdot v^n.$$

45) $$k_n = \frac{g_0 + h_0 + 2k_0}{2^{n+1}} - \frac{g_0 + h_0}{2} \cdot v^n.$$

46) $$l_n = \frac{g_0 + h_0 + 2l_0}{2^{n+1}} - \frac{g_0 + h_0}{2} \cdot v^n.$$

From equation 42) we get

$$h_n = v^n(g_0 + h_0) - g_n.$$

Substituting this value of h_n into equation 36) and simplifying we have,

$$g_n = [2(r^2-1)g_{n-1} + 2v^{n-1}(g_0 + h_0)]/R.$$

If we let $(r^2-1)/2(r+1)^2 = w$, this equation takes the simpler form,

$$g_n - w\,g_{n-1} = (g_0 + h_0)v^{n-1}/2(r+1)^2.$$

The solution is,

47) $$g_n = (g_0 - h_0)w^n/2 + (g_0 + h_0)v^n/2.$$

Substituting for g_n from equation 47) into equation 42) we have

48) $$h_n = (h_0 - g_0)w^n/2 + (g_0 + h_0)v_n/2.$$

We can now evaluate everything in equation 32) excepting c_n and c_{n-1} and have,

$$c_n - c_{n-1} = \frac{g_0 - h_0}{4} w^n + \frac{g_0 + h_0}{4}(v^n - v^{n-1}) + \frac{g_0 + h_0 + i_0 + j_0}{2^{n+1}}.$$

The solution is

49) $$c_n = \frac{g_0 - h_0}{4} \cdot w\frac{(1-w^n)}{1-w} + \frac{g_0 + h_0}{4}(v^n - 1) + (g_0 + h_0 + i_0 + j_0)\left(\frac{2^n - 1}{2^{n+1}}\right) + c_0.$$

Similarly,

50) $$d_n = \frac{h_0 - g_0}{4} \cdot w\frac{(1-w^n)}{1-w} + \frac{g_0 + h_0}{4}(v^n - 1) + (g_0 + h_0 + i_0 + k_0)\left(\frac{2^n - 1}{2^{n+1}}\right) + d_0.$$

51) $$e_n = \frac{h_0 - g_0}{4} \cdot w\frac{(1-w^n)}{1-w} + \frac{g_0 + h_0}{4}(v^n - 1) + (g_0 + h_0 + j_0 + l_0)\left(\frac{2^n - 1}{2^{n+1}}\right) + e_0.$$

52) $$f_n = \frac{g_0 - h_0}{4} \cdot w\frac{(1-w^n)}{1-w} + \frac{g_0 + h_0}{4}(v^n - 1) + (g_0 + h_0 + k_0 + l_0)\left(\frac{2^n - 1}{2^{n+1}}\right) + f_0.$$

DISCUSSION. 1. It is easy to get the limiting population in this problem. Since v and w are proper fractions, v^n and w^n approach zero as n increases. Because of this, *the limits are zero for all but the homozygous types, c, d, e, f. For these four we have,*

$$\lim_{n=\infty} c_n = (g_0 - h_0)(r+1)/2(r+3) + (h_0 + i_0 + j_0)/2 + c_0.$$

$$\lim_{n=\infty} d_n = (h_0 - g_0)(r+1)/2(r+3) + (g_0 + i_0 + k_0)/2 + d_0.$$

$$\lim_{n=\infty} e_n = (h_0 - g_0)(r+1)/2(r+3) + (g_0 + j_0 + l_0)/2 + e_0.$$

$$\lim_{n=\infty} f_n = (g_0 - h_0)(r+1)/2(r+3) + (h_0 + k_0 + l_0)/2 + f_0.$$

In all the one-factor problems in self-fertilization or any other forms of inbreeding that have been discussed by JENNINGS (1916) *and by the present writer* (ROBBINS 1917, 1918) *the heterozygous type tends to disappear. Here in the two-factor problem in self-fertilization we note the same tendency.*

2. In general the proportions in the limiting population depend upon the linkage factor r, but in case $h_0 = g_0$, i.e., when the two types ABab and AbaB appear in equal numbers, the limiting population is independent of the linkage factor.

b. Linkage r in one set of gametes and r′ in the other set

The recurrence relations for this more general problem may be obtained by replacing r^2 by rr' and $2r$ by $r+r'$ in equations 32) to 41) above. The solutions have the same form as above, equations 43) to 52) but v and w have the values

$v = (rr' + 1)/2(r+1)(r'+1)$; $w = (rr' - 1)/2(r+1)(r'+1)$. The limiting population takes the form obtained by replacing $(r+1)/2(r+3)$ in the previous limiting forms by $(rr' - 1)/(rr' + 2r + 2r' + 3)$.

DISCUSSION. 1. In case of no linkage in either set of gametes, $r = r' = 1$, the equations simplify considerably since $w = 0$ and $v = \frac{1}{4}$.

2. In case of no linkage in one set of gametes and linkage r in the other set, $r' = 1$, we have $v = \frac{1}{4}$; $w = (r-1)/4(r+1)$.

3. In case of complete linkage in both sets of gametes, $r = \infty$, we have $w = v = \frac{1}{2}$. The value of each class except those which are homozygous reduces to its original value divided by 2^n. The homozygous classes have the values,

$$c_n = (g_0 + i_0 + j_0)\left(\frac{2^n - 1}{2^{n+1}}\right) + c_0 ,$$

$$d_n = (h_0 + i_0 + k_0)\left(\frac{2^n - 1}{2^{n+1}}\right) + d_0 ,$$

$$e_n = (h_0 + j_0 + l_0)\left(\frac{2^n - 1}{2^{n+1}}\right) + e_0 ,$$

$$f_n = (g_0 + k_0 + l_0)\left(\frac{2^n - 1}{2^{n+1}}\right) + f_0 .$$

4. In case of complete linkage in one set of gametes and r in the other we have $v = w = r/2(r + 1)$.

5. In case of complete linkage in one set of gametes and no linkage in the other set, $v = w = 1/4$.

LITERATURE CITED

JENNINGS, H. S., 1916 The numerical results of diverse systems of breeding. Genetics **1**: 53-89.

1917 The numerical results of diverse systems of breeding, with respect to two pairs of characters, linked or independent, with special relation to the effects of linkage. Genetics **2**: 97-154.

ROBBINS, R. B., 1917 Some applications of mathematics to breeding problems. Genetics **2**: 489-504.

1918 Applications of mathematics to breeding problems II. Genetics **3**: 73-92.

RANDOM MATING WITH THE EXCEPTION OF SISTER BY BROTHER MATING

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In tracing the fate of a Mendelian character, the results for random mating are well known. The results for brother and sister mating have been published. It is at once seen to be of interest to know what will be the results for mating at random excepting that brothers shall not mate with sisters. This paper deals with the problem just stated. For the reader who does not care to follow the details of the discussion, the principal results are included in a summary.

We assume that all families have the same number of individuals, equally divided between males and females and that the division as to dominant (A) and recessive (a) characters is independent of the division as to sex.

Let each family consist of $4k$ male and $4k$ female children, $4k$ being an integer. It is necessary to consider the types of families which arise from different crosses. These are tabulated below.

Type of cross	Composition of resulting family (AA, Aa, aa)	Letter indicating type of family and number of families of this type
$AA \times AA$	$(8k, 0, 0)$	p
$AA \times Aa$	$(4k, 4k, 0)$	q
$AA \times aa$	$(0, 8k, 0)$	r
$Aa \times Aa$	$(2k, 4k, 2k)$	s
$Aa \times aa$	$(0, 4k, 4k)$	t
$aa \times aa$	$(0, 0, 8k)$	u

It seems essential to express the numbers of families of the various types in one generation in terms of the corresponding numbers for the preceding generation. For this purpose we will use capital letters when

referring to one generation and small letters when referring to the preceding generation. If we let

$$f = p + q + r + s + t + u,$$

a study of the possible crosses enables us to write down the following recurrence relations:

$$1)\quad \begin{cases} P = \dfrac{k}{f-1}\left[4pq + 2ps + 4p(p-1) + q(q-1) + \tfrac{1}{4}s + \tfrac{1}{4}s(s-1)\right] \\ Q = \dfrac{k}{f-1}\left[4pq + 8pr + 4ps + 4pt + 2q(q-1) + 4qr + 3qs + 2qt + 2rs + s(s-1) + st\right] \\ R = \dfrac{k}{f-1}\left[2ps + 4pt + 8pu + qs + 2qt + 4qu + \tfrac{1}{2}s(s-1) + st + 2us\right] \\ S = \dfrac{k}{f-1}\left[q(q-1) + 4qr + 2qs + 2qt + 4r(r-1) + 4rt + 4rs + s(s-1) + 2st + t(t-1)\right] \\ T = \dfrac{k}{f-1}\left[qs + 2qt + 4qu + 2rs + 4rt + s(s-1) + 3st + 4us + 2t(t-1) + 8ru + 4ut\right] \\ U = \dfrac{k}{f-1}\left[\tfrac{1}{4}s(s-1) + st + 2us + t(t-1) + 4ut + 4u(u-1)\right] \end{cases}$$

It is from these equations that we must derive whatever information we get regarding the outcome of the type of mating under consideration. The complicated nature of the equations makes a direct solution of them seem hopeless. However, certain combinations of them lead to hopeful simplifications and finally yield much desired information. If we add the six equations just as they stand and use the notation

$$F = P + Q + R + S + T + U,$$

we have

$$F = 4kf$$

A moment's thought shows that this must be the case, i.e., if each family has 8 k children, the number of families in one generation will be 4 k times the number in the preceding generation, it being assumed that one male mates with one female. If f_n is the number of families in the nth generation we have at once

2) $f_n = f_0(4\,k)^n$.

Let a, b, c be proportional respectively to the number of AA, Aa and aa individuals in a generation. The table giving the composition of the various types of families enables us to choose a, b, c as follows:

$$3)\quad \begin{cases} a = 2\,p + q + s/2, \\ b = q + 2\,r + s + t, \\ c = s/2 + t + 2\,u. \end{cases}$$

We note that $a + b + c = 2\,f$. By using equations 3) we are able to put equations 1) in a simpler form:

$$4)\quad \begin{cases} P = \dfrac{k}{f-1}\,[a^2 - a - 2\,p + s/4], \\ Q = \dfrac{k}{f-1}\,[2\,a\,b - 2\,a + 4\,p], \\ R = \dfrac{k}{f-1}\,[2\,a\,c - s/2], \\ S = \dfrac{k}{f-1}\,[b^2 - b - 2r], \\ T = \dfrac{k}{f-1}\,[2\,b\,c - 2\,c + 4\,u], \\ U = \dfrac{k}{f-1}\,[c^2 - c - 2\,u + s/4]. \end{cases}$$

If one wishes to use recurrence relations for the purpose of calculating the numbers for one generation from those for the preceding generation, equations 4) are much more easily used than equations 1). By thinking of equations 3) as written in capital letters, we can use equations 4) to obtain A, B, C in terms of the small letters thus:

$$5)\quad \begin{cases} A = \dfrac{k}{2(f-1)}\,[(b + 2a)^2 - 8a - b - 2r + s], \\ B = \dfrac{k}{f-1}\,[(b + 2a)(b + 2c) - 3b + 2r - s], \\ C = \dfrac{k}{2(f-1)}\,[(b + 2c)^2 - 8c - b - 2\,r + s]. \end{cases}$$

Equations 5) are more compact than equations 4), but give us less information; in fact we could not use equations 5) in calculating the numbers for succeeding generations because they do not give us R and S and these would be needed in the next step. However, if we combine with equations 5) the following pair from equations 4),

$$R = \frac{k}{f-1}[2\,ac - s/2],$$

$$S = \frac{k}{f-1}[b^2 - b - 2r],$$

we have a set better adapted for calculating the numbers for successive generations than is set 4).

Combining equations 4) we find that

$$6)\quad \begin{cases} 2P + Q + R = 4k\,a, \\ 2U + T + R = 4k\,c, \\ 2S + Q + T = 4k\,b. \end{cases}$$

Here we have a set of recurrence relations solved for the variables which, if we were using subscript notation, would have lower subscripts. The equations are useless for direct computation but they will soon become important in an indirect way.

From equations 5) we find that

$$7)\quad \begin{cases} B + 2A = 4k\,(b + 2a), \\ B + 2C = 4k\,(b + 2c), \end{cases}$$

Whence,

$$8)\quad \begin{cases} \dfrac{B + 2A}{B + 2C} = \dfrac{b + 2a}{b + 2c}. \end{cases}$$

The ratio of dominant to recessive gametes in a particular generation is $(b + 2a)/(b + 2c)$. Equation 8) shows that this ratio is the same in one generation as in the preceding generation; from this we deduce immediately that the ratio of dominant to recessive gametes combining to form the individuals of the various generations is constant. This is a significant fact in that it is also true for complete random mating and for brother and sister mating. Using subscript notation, the solutions of system 7) are

$$9)\quad \begin{cases} b_n + 2a_n = (b_0 + 2a_0)(4k)^n. \\ b_n + 2c_n = (b_0 + 2c_0)(4k)^n. \end{cases}$$

By means of equations 3), equations 6) may be rewritten with subscripts thus:

$$10)\quad \begin{cases} r_n - s_n/2 = 4k\,a_{n-1} - a_n, \\ r_n - s_n/2 = 4k\,c_{n-1} - c_n, \\ -2r_n + s_n = 4k\,b_{n-1} - b_n. \end{cases}$$

In the light of 9) and 10), equations 5) may be written with subscripts as follows:

$$11)\quad \begin{cases} a_n = \dfrac{k}{f_0(4k)^{n-1}-1}\left[\tfrac{1}{2}(b_0+2a_0)^2(4k)^{2n-2}-\tfrac{1}{2}(b_0+2a_0)(4k)^{n-1}\right. \\ \qquad\qquad \left.-2a_{n-1}-4ka_{n-2}\right], \\ b_n = \dfrac{k}{f_0(4k)^{n-1}-1}\left[(b_0+2a_0)(b_0+2c_0)(4k)^{2n-2}-2\,b_{n-1}\right. \\ \qquad\qquad \left.-4\,k\,b_{n-2}\right], \\ c_n = \dfrac{k}{f_0(4k)^{n-1}-1}\left[\tfrac{1}{2}(b_0+2c_0)^2(4k)^{2n-2}-\tfrac{1}{2}(b_0+2c_0)(4k)^{n-1}\right. \\ \qquad\qquad \left.-2\,c_{n-1}-4k\,c_{n-2}\right]. \end{cases}$$

The solutions of this set of equations would give us a_n, b_n, c_n in terms of the original data.

It is well now to distinguish two cases. Consider first the case in which there are just two children in each family; in this case, $4k = 1$. Then equations 11) simplify considerably, giving us,

$$12)\quad \begin{cases} 8(f_0-1)a_n + 4a_{n-1} + 2a_{n-2} = (b_0+2a_0)(b_0+2a_0-1), \\ 4(f_0-1)b_n + 2b_{n-1} + b_{n-2} = (b_0+2a_0)(b_0+2c_0), \\ 8(f_0-1)c_n + 4c_{n-1} + 2c_{n-2} = (b_0+2c_0)(b_0+2c_0-1). \end{cases}$$

The solutions are

$$13)\quad \begin{cases} a_n = \rho^n\left[k_1 \cos n\theta + k_2 \sin n\theta\right] + \dfrac{(b_0+2a_0)(b_0+2a_0-1)}{8f_0-2}, \\ b_n = -2\rho^n\left[k_1 \cos n\theta + k_2 \sin n\theta\right] + \dfrac{2(b_0+2a_0)(b_0+2c_0)}{8f_0-2}, \\ c_n = \rho^n\left[k_1 \cos n\theta + k_2 \sin n\theta\right] + \dfrac{(b_0+2c_0)(b_0+2c_0-1)}{8f_0-2}, \end{cases}$$

in which $1/\rho = 2\sqrt{f_0-1}$ and $\tan\theta = -\sqrt{4f_0-5}$.

Aside from the value of these equations for purposes of computation, they give us the limiting values of a_n, b_n, c_n as n increases indefinitely. In other words we get the final results for this type of mating if indefinitely continued. If we denote the limiting values of a_n, b_n, c_n, as n increases indefinitely, by a', b', c' we have

$$a' = \frac{(b_0+2a_0)(b_0+2a_0-1)}{8f_0-2} \qquad b' = \frac{2(b_0+2a_0)(b_0+2c_0)}{8f_0-2},$$

$$c' = \frac{(b_0+2c_0)(b_0+2c_0-1)}{8f_0-2}$$

For completely random mating, the proportions of the three types are fixed after the first random mating and are[1]

$$a' : b' : c' : : (b_0 + 2a_0)^2 : 2(b_0 + 2a_0)(b_0 + 2c_0) : (b_0 + 2c_0)^2.$$

In brother and sister mating, the heterozygous type tends to disappear and for a limiting population we have (ROBBINS 1917)

$$a' : c' : : (b_0 + 2a_0) : (b_0 + 2c_0).$$

By comparing these three sets of results we note that omitting the brother and sister mating has had the effect of increasing the proportion of heterozygous individuals over what it is in completely random mating, *when there are only two offspring in a family.*

Consider now the case in which $4k > 1$, i.e., the case in which there are more than two offspring to a family. Returning to equations 11), it would be desirable to solve these equations without restrictions on k if possible. However, the nature of the solutions of the simple case in which $4k = 1$ seems to indicate that probably the solution of the general case would be of little use for purposes of computation even if we had it. We can get all desirable information about the limiting population without solving equations 11). We can calculate immediately from equations 11) that if $4k > 1$,

$$\lim_{n=\infty} \frac{a_n}{(4k)^n} = \frac{(b_0 + 2a_0)^2}{8 f_0},$$

$$\lim_{n=\infty} \frac{b_n}{(4k)^n} = \frac{2(b_0 + 2c_0)(b_0 + 2a_0)}{8 f_0},$$

$$\lim_{n=\infty} \frac{c_n}{(4k)^n} = \frac{(b_0 + 2c_0)^2}{8 f_0}.$$

Then using a', b', c' for the limiting values as above,

$$a' : b' : c' : : (b_0 + 2a_0)^2 : 2(b + 2a_0)(b_0 + 2c_0) : (b_0 + 2c_0)^2.$$

This is the same result as in random mating. Thus in case a typical family consists of more than two offspring, the effect of omitting brother and sister mating from otherwise random mating is only temporary, the final results being the same as for completely random mating.

SUMMARY

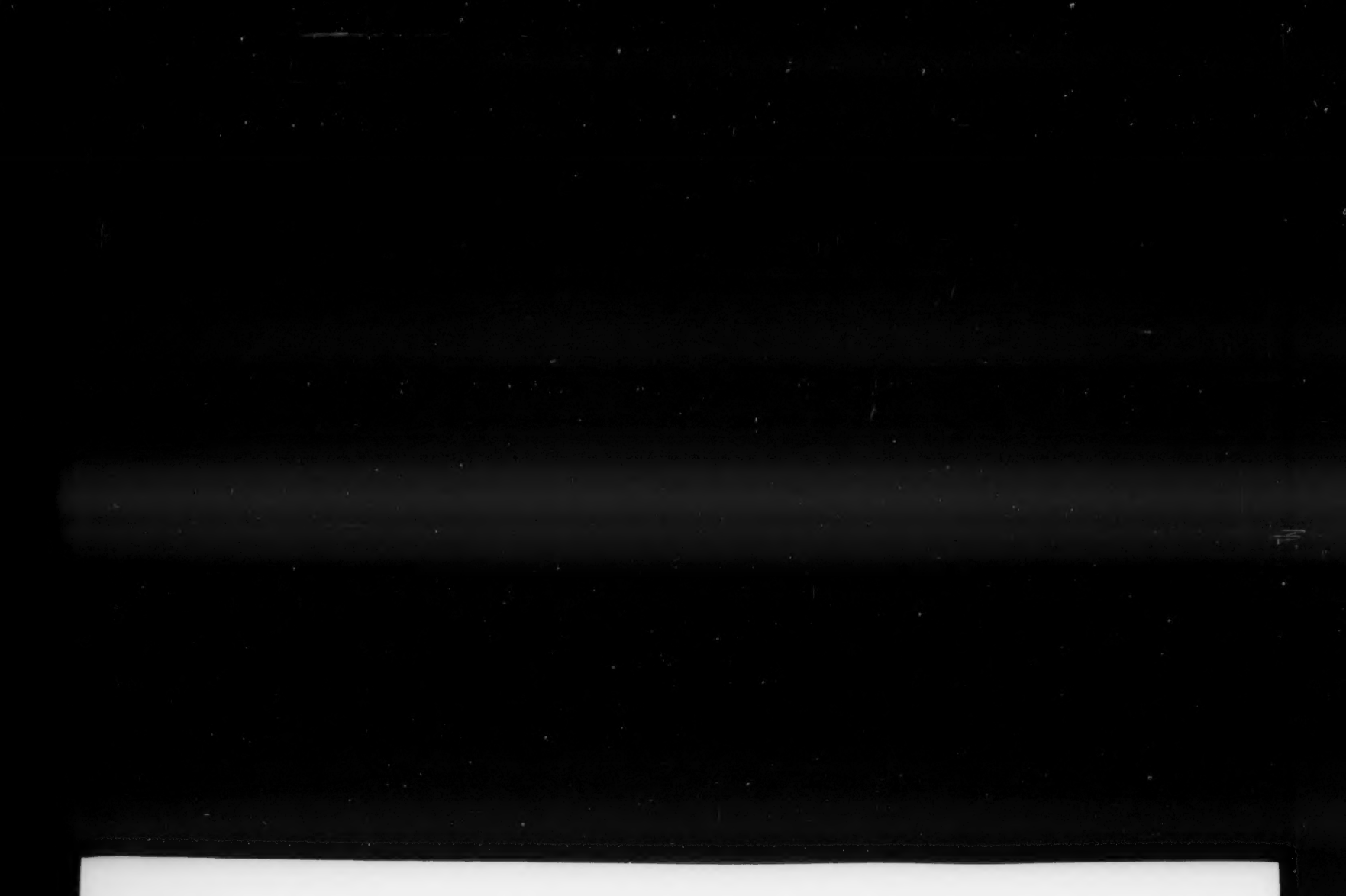
This paper is a discussion of the problem of random mating with the exception that brothers shall not mate with their sisters, a single pair of Mendelian factors being considered. Three principal results are accomplished. First, formulae are developed from which we can calculate

[1] See for instance WENTWORTH and REMICK (1916).

the distribution of the offspring for succeeding generations (a) as to type of family,—equations 3) and 4)—and (b) as to type of individual, —equations 5). Second, in case a family consists of two offspring the problem is solved completely in equations 13). Third, the distribution of the limiting population is given in case there are two offspring in a family and also in case there are more than two offspring in a family. The most important conclusions may be stated thus:

When brother and sister mating is omitted from otherwise random mating, the progeny in succeeding generations approaches a fixed distribution as the number of generations increases, in which pure dominants, heterozygotes and recessives all appear. (a) In case there are but two offspring in a typical family, the proportion of heterozygotes in the limiting population is greater than for completely random mating. (b) In case there are more than two offspring in a typical family, the limiting population will be the same as if the mating had been completely random.

LITERATURE CITED

WENTWORTH, E. N., and REMICK, B. L., 1916 Some breeding properties of the generalized Mendelian population. Genetics **1**: 608-616.

ROBBINS, R. B., 1917 Some applications of mathematics to breeding problems. Genetics **2**: 489-504.